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STUDIES OF SOIL MOISTURE IN THE "GREAT PLAINS" REGION.

BY F. J. ALWAY, PH.D.

Professor of Agricultural Chemistry, University of Nebraska.

THE "Great Plains" region, the western portion of what is commonly known as the prairies of North America, extends from the northern parts of Texas and New Mexico into the south of Alberta and Saskatchewan. It forms a belt stretching from the foothills of the Rocky Mountains eastward for some two or three hundred miles, the eastern boundary being ill-defined and fluctuating with the cycles of wet and dry seasons. The region is characterised by a dry atmosphere with strong winds, low minimum temperatures in the winter and very high maxima in the summer, with a comparatively small rainfall, most of which occurs during the summer months. A similar total precipitation of 15 or 16 inches per annum occurs west of the Rocky Mountains, but falls chiefly during the winter months and is then found to be sufficient for the profitable production of grain crops. In the "Great Plains" region in Saskatchewan the usual custom is to take a bare fallow one season in three in order to accumulate the rainfall for the benefit of the two following grain crops.

This rough rule, which does not take into account the variable character of the seasons, can advantageously be replaced by determinations of the water content of the soil before the growing season commences, so as to measure the amount of water available for the crop.

Before however any judgement can be formed on this point it is necessary (1) to take samples down to the depth of six feet, (2) to determine not merely the total water but the amount of "free" water in the soil.

As to the depth to which samples should be taken it is found that in Saskatchewan on the clay soil generally the roots of cereals and grasses penetrate to the depth of four to six feet; below this depth

the limited rainfall is rarely likely to penetrate nor will the moisture from the lower layer be elevated by capillarity to any effective extent. By the "free" water in the soil is understood the measured water less the "hygroscopic coefficient" of the soil as defined by Hilgard[1], *i.e.* the percentage of moisture which a thin layer of soil can absorb from an atmosphere kept saturated with water vapour. It is certain that plants cannot utilise any of the hygroscopic water in a soil for growth, though data are lacking as to how near this point the soil water can be reduced without killing the plant; at any rate the "free" water measures the maximum amount that can be available for the plant. As the "hygroscopic coefficient" of the soils of this region examined by the author varies between 4 and 14 it is obvious that determinations only of the water present in the soil would give very erroneous measures of the relative amounts of water available for the crop.

The author's observations were made in July 1904 and Sept. 1905 on the Canadian Experimental Farm at Indian Head, and at Moose Jaw, Saskatchewan. The samples were taken with an auger, 1½ inches in diameter; they were weighed in the field and then despatched to the laboratory in Nebraska for determination of the dry matter and the "hygroscopic coefficient."

1. *Uniform soil—effect of crop v. fallow.*

Soil samples were taken on Sept. 9th, 1905 from two adjoining fields at Moose Jaw, one being under an unusually clean summer fallow, the other having just yielded 40 bushels of wheat and 3200 lbs. of straw per acre. In 1903 both fields had been under small grain crops, and in 1904 the first field had been fallowed while the second had carried oats or wheat. In 1904 the rainfall had only been 7·90 inches, but in 1905 as much as 15·32 inches had already fallen, of which only 0·78 inch had fallen between July 30th of that year and the date of sampling.

Table I. shows both total and free water in each successive foot of soil down to the depth of six feet: the soil is a lacustral clay of a heavy type, the mechanical analysis of which is given in Table II. Determinations of the "hygroscopic coefficient" and of the carbon dioxide evolved on treatment with acid of these and other samples show its uniformity over a considerable area.

[1] University of California Agric. Exp. Sta., *Circular* 6 (1903), p. 17.

TABLE I.

Water in fields on uniform lacustral clay.

	Foot section	Percentage of total water	Hygroscopic coefficient	Percentage of free water	Field notes on condition of soil
Field G, Fallow	First	23·6	13·9	9·7	Moist
	Second ...	36·8	12·2	24·6	Very moist
	Third.....	33·2	11·7	21·5	,, ,,
	Fourth ...	27·7	11·8	15·9	Moist
	Fifth	26·0	11·9	14·1	,,
	Sixth	25·2	11·5	13·7	,,
	Average ...	28·7	12·1	16·6	
Field F, Wheat	First	22·2	13·6	8·6	Moist
	Second ...	18·6	12·7	5·9	Dry
	Third.....	18·0	11·8	6·2	,,
	Fourth ...	23·5	11·9	11·6	Moist
	Fifth	24·4	11·7	12·7	,,
	Sixth	25·0	11·4	13·6	,,
	Average ...	21·9	12·2	9·8	

The fallow field G contained within the first six feet of soil an average of 6·8 per cent. more water than the adjoining field F, which had just brought a crop of wheat to maturity. The wheat roots had not drawn as heavily upon the moisture of the fourth and fifth feet as upon that of the second and third feet, nor had the season's rainfall been sufficiently conserved to raise the moisture content of the soil below the third foot to near its limit. It should be noted, however, that the season of 1905 with an exceptionally well distributed rainfall had not been favourable for the reduction of the soil moisture to its minimum, and also that the rainfall of the previous year had not been sufficiently heavy at Moose Jaw to raise the moisture content of the first four feet to its maximum, even if surface evaporation had been entirely prevented. In the case of the fallow field about half of the rainfall of the season had been stored, chiefly below the first foot.

When the amounts of total water in the fields, F and G, are compared, the relative moisture conditions are shown correctly, thus rendering it not necessary to make determinations of the hygroscopic coefficient on all the different samples when only adjacent fields are under comparison. The necessity of deep sampling, however, is very apparent. If samples had been taken to a depth of only one foot,

very little difference in the moisture content of the two fields would have been found, although there was, in reality, enough difference to account for a difference in yield in the following year, as shown by another experiment on the same farm, of twenty bushels of wheat per acre.

TABLE II.

Mechanical analysis of specimens of the two soil types.

	Boulder clay per cent.	Lacustral clay per cent.
Gravel, 2·0 to 1·00 mm.	1·7	0·0
Coarse sand, 1·0 to 0·5 mm.	5·2	0·01
Medium sand, 0·5 to 0·25 mm.	5·6	0·01
Fine sand, 0·25 to 0·10 mm.	14·1	2·2
Very fine sand, 0·10 to 0·05 mm.	12·3	6·7
Coarse silt, 0·05 to 0·016 mm.	16·2	19·0
Fine silt, 0·016 to 0·005 mm.	19·5	22·6
Clay, below 0·005	26·1	48·2
Total	100·7	98·8

2. *Mixed soil.*

In the fields of the Indian Head Experimental Farm from which the following samples were taken two types of soil are to be found; a lacustral clay in every way similar to that described at Moose Jaw forms the surface layer and possesses a thickness varying from a few inches to three feet, below which it is underlaid by a boulder clay. The latter soil, as shown by the mechanical analysis in Table II., is of much lighter character, it contains less clay and more carbonate of lime, and possesses a much lower hygroscopic coefficient, 4—7 °/₀ instead of 11—15 °/₀ for the lacustral clay.

In Table III. the water content of the soils from five different fields is brought together for comparison, samples having been taken both in 1904 and 1905; only the total water and the free water figures are shown, the latter being printed in italics, it being understood that in each case the hygroscopic coefficient has been determined and deducted from the total water in order to arrive at the free water.

During 1904 the summer rainfall had been light, amounting only to 6·67 inches between April 1st and the time of sampling, and of this 1·15 inch had fallen on July 12th, 13th shortly before sampling. During 1905 the rainfall up to the end of August had been 13·77 inches, of which 1·28 inch fell on Aug. 30th, 31st, immediately before sampling.

TABLE III.

Water in soils of mixed origin at Indian Head.

Foot Section	D		B		A		C		E	
	Fallow		Fallow		Grass		Oats		Fallow	
	Total	Free	Total	Free	Total	Free	Total	Free	Total	Free
July 25th, 1904										
First	34·4	*20·9*	29·4	*17·4*	19·0	*7·5*	20·0	*7·3*		
Second ...	29·7	*16·2*	14·9	*11·0*	14·8	*4·5*	22·4	*9·2*		
Third ...	29·5	*16·6*	16·4	*11·7*	13·9	*5·8*	21·6	*8·1*		
Fourth ...	20·3	*12·9*	17·4	*11·9*	13·6	*6·7*	16·1	*11·5*		
Fifth	15·2	*8·6*	21·8	*14·2*	12·0	*5·0*	15·1	*10·9*		
Sixth	19·2[1]	*12·6*	19·6	*11·6*	15·4	*6·5*	15·9	*10·0*		
Mean ...	24·7	*14·6*	19·9	*12·9*	14·8	*6·0*	18·5	*9·5*		
September 5th, 1905										
	Wheat		Oats		Grass		Wheat		Wheat	
First	28·9	*16·1*	22·4	*10·6*	25·9	*15·9*	28·8	*17·9*	26·4	*16·9*
Second ...	22·7	*9·6*	16·7	*5·9*	20·3	*9·4*	19·8	*10·1*	14·8	*7·9*
Third.....	20·9	*8·6*	9·2	*4·5*	20·9	*10·0*	10·9	*6·1*	15·4	*9·6*
Fourth ...	11·8	*6·3*	9·6	*5·2*	15·4	*10·7*	17·1	*11·7*	13·2	*7·4*
Fifth	16·4	*10·9*	14·4	*10·0*	15·1	*10·2*	18·4	*12·9*	15·6	*10·0*
Sixth	19·2	*12·6*	17·8	*12·6*	13·6	*9·1*	19·0	*14·6*	21·8	*11·8*
Mean ...	19·9	*10·6*	15·0	*8·1*	18·5	*10·9*	19·0	*12·2*	17·9	*10·6*

The determinations of hygroscopic coefficient indicate that the lacustral clay extended to a depth of three feet in field D, a foot only in field B, about two feet in A, and about three feet in C, while the samples from field E were taken from a ridge of the boulder clay.

In field D the determinations in 1904 show plenty of free water under the fallow; in 1905 despite the growth of a crop of 32 bushels of grain and 3700 lbs. of straw per acre, the subsoil had been by no means completely exhausted of its free water. The moistness of the first two feet was due to the rains subsequent to the cutting of the crop.

Field B gave very similar results to D; uniformly moist under the fallow in 1904, it has been considerably but not completely dried by the oat crop of 1905 (95·3 bushels of grain and 5960 lbs. of straw per

[1] This sample was lost, so the figure for the sixth foot in 1905 has been adopted.

acre) in the second, third and fourth feet, but the fifth and sixth feet were still moist and the surface had regained moisture by the recent rains.

Field A was covered with Western Rye Grass (*Agropyrum tenerum*) and was cut for hay on July 18th, 1904.

During this dry season the grass had very thoroughly exhausted the soil of water; down to the sixth foot there was always less than 7 per cent. of free water, the slight excess in the top foot being due to the rainfall of July 12th, 13th. Roots were found to a depth of 5′ 6″, and in the sixth foot while the upper layers of soil were quite dry and powdery the lowest layer was moist and sticky. In 1905 it is clear that but little of the rainfall after mowing on July 13 had been transpired by the grass, so that the subsoil down to six feet had become moist.

Field C, which adjoins D, is continuously cropped with cereals, and at the time of sampling in 1904 was showing the effects of the drought more than any other field on the farm, the straw being short and yellow. Notwithstanding this the soil appeared moist at all depths and showed more free water than several of the other fields.

When these results are considered together it is seen that the total amounts of water in the soil would lead to very erroneous judgements as to its capacity for supporting a crop. For example field F in 1905, which had just brought a heavy crop of wheat to maturity after a month of almost rainless weather, held more water in the first six feet of soil than did B in 1904, after a season's fallowing. Field C also in 1904, though showing serious effects of drought, contained only 1·4 per cent. less moisture than did B on the same day. Yet in the following year the yield of grain and straw per acre was 9200 lbs. on B and 2160 lbs. only on C.

When the fields are arranged in the order of their amount of free water they are given more nearly their proper place, all the fallows being placed before the grain and grass fields.

A comparison of the fields on the basis of the amount of free water in the first six feet assumes that a definite value is to be attached to a certain percentage of free water, independent of the kind of soil and of the portion of the six feet in which it occurs. It is evident from Hilgard's work, however, that a higher percentage of free moisture is required on clay than on sandy soils in order that plant growth may continue. The data in the above tables indicate that wheat and oats may reduce the free water to about 6 per cent. through

the first three feet of lacustral clay and to 4 or 5 per cent. through the first four feet of boulder clay. The fourth and fifth feet, respectively, while not being exhausted to the same extent as the soil above, give up part of their free water. The fifth and sixth feet, respectively, seem to furnish a small and, perhaps, negligible portion of their water to oats and wheat. In the case of the boulder clay, a native grass is able to exhaust the fifth and the upper half of the sixth foot as completely as the two cereal crops do the fourth.

In Table IV. an attempt has been made to show, as well as the limited amount of data will permit, the quantities of water, in the different fields, available for wheat and oat crops. It has been assumed that the lower limit of free moisture in the boulder clay is 4·5 and in the lacustral clay 6 per cent. The free water less this 4·5 or 6 per cent. is for convenience at present designated the "x water." It has also been assumed that all the water of the first three feet and half of that of the fourth foot are available in the fields at Moose Jaw and that the x water of four and a half feet is available at Indian Head.

If the fields are arranged in the order of the amount of x water within reach of wheat and oat roots, and if they are also ranked according to the writer's field notes concerning the moistness or otherwise of each soil section, it is interesting to find that both methods show the fallows grouped at the top and the three driest fields at the bottom. Thus the judgement of an agriculturist formed without the use of any apparatus except an auger is more reliable than a determination of total moisture when the hygroscopic coefficient is not also determined.

The ordinary prairie farmer, provided with a six foot auger, can form a fair estimate of the moisture condition of his fields before the spring is sufficiently advanced to permit of seeding, and will thus be in a position to decide intelligently whether to sow grain upon his stubble fields or to summer-fallow them. At present his decision is governed by the rule "one year of fallow followed by two years of grain." Normally the soil is dry enough in the spring to justify the rule, but in such years as 1899 and 1901, when the preceding autumn had been exceptionally wet (nine inches of rain falling after the ripening of the cereal crops), the stubble fields have probably been nearly as wet to a depth of five feet as the fallows were.

TABLE IV.

The amount of water in different fields that is probably available for the support of normal growth of wheat and oats, expressed as percentage of dry soil.

Foot section	D July 1904	G Sept. 1905	B July 1904	C Sept. 1905	A Sept. 1905	E Sept. 1905	D Sept. 1905	C July 1904	B Sept. 1905	F Sept. 1905	A July 1904
First	14·9	3·7	11·4	11·9	9·9	10·9	10·1	1·3	4·6	2·6	1·6
Second	10·2	18·6	6·5	4·1	3·4	3·4	3·6	3·2	1·4	0·0	0·0
Third	10·6	15·5	7·2	1·6	4·0	5·1	2·6	2·1	0·0	0·2	1·3
Fourth	8·4	4·9	7·4	7·2	6·2	2·9	1·8	7·0	0·7	2·8	2·2
Fifth	2·1	0·0	4·8	4·2	2·8	2·7	3·2	3·2	2·2	0·0	0·2
Average of 5 feet	9·2	8·5	7·5	5·8	5·3	5·0	4·3	3·4	1·8	1·1	1·1
Tons per acre ...	690	637	562	435	397	375	325	255	135	83	83
Inches of Rain...	6·1	5·6	5·0	3·9	3·5	3·3	2·9	2·2	1·2	0·6	0·6

3. *The depth to which the frost penetrates.*

Both English and American authorities have advanced or accepted, in explanation of the large yields of grain in southern Saskatchewan, the theory that the frost penetrates to a great depth during the winter, and during the summer months furnishes a constant supply of moisture to the growing grain.

Sir William Crookes[1] has stated:

"The fertility of the North-west Provinces of the Dominion of Canada is due to an exceptional and curious circumstance. In winter the ground freezes to a considerable depth. Wheat is sown in the spring, generally April, when the frozen ground has been thawed to a depth of three inches. Under the hot sun of the short summer the grain sprouts with surprising rapidity, partly because the grains are supplied with water from the thawing depths. The summer is too short to thaw the ground thoroughly, and gate posts or other dead wood extracted in the autumn are found still frozen at their lower ends." Storer[2] in his textbook on Agriculture quotes, as an illustration

[1] Presidential Address, British Association (1898), Report, p. 8; *The Wheat Problem*, p. 22 (1900).

[2] *Agriculture* (7th Ed.), vol. I. p. 144.

of how melting ice may serve as a useful store of moisture in certain situations and for certain crops, that in Assiniboia[1], "even in late July some of the soil still holds the winter's frost at a depth of several feet below the surface. This underground layer of frozen earth is believed to explain the wonderful fertility of the soil, as the frost, in gradually coming to the surface during the summer months, creates a moisture which, meeting the warmth from above, forms a kind of natural hot bed. This moisture counteracts the scarcity of rain during the spring and summer and accounts for the grain being forced with such amazing rapidity after the late sowing."

The winter of 1903—1904 was the coldest that had occurred in twelve years in Saskatchewan, the spring was late, and the early part of the summer cool. During the second week in July 1904, the writer made borings to a depth of nine feet in the most exposed situations to be found in the vicinity of Moose Jaw. There was no trace of frost, although the soil, at a depth of six feet, was distinctly chilly to the touch; both above and below it was decidedly warmer. In the same district on the 1st of April in 1906 numerous borings were made through the frozen soil. In the exposed gardens and fallows the frost had penetrated from 4½ feet to 5½ feet. On the stubble it had reached a depth of only 3½ feet.

Not even the chilly layer of subsoil was found in the borings made at Chaplin and at Indian Head in 1904 or at the latter place and Moose Jaw in September 1905.

It is probable that the frost is all out of the ground before the 1st of July of every summer, unless it be on the edge of the forest country, where the drifting leaves may serve to prevent the thawing of the soil.

It is possible that the low winter temperature may assist in the transfer of water, by distillation, from the lower moist zone to the upper, if the latter has been dried out by a crop. The frost, however, seems to have nothing to do with the exceptionally large yields of grain which are obtained only on summer-fallowed land or its equivalent, and which bear no relation to the temperature of the preceding winter.

SUMMARY AND CONCLUSIONS.

1. All determinations of soil moisture should be made to a depth of from four to five feet for wheat and oats, and to a depth of six or seven feet for grasses.

[1] Now the southern portion of the Province of Saskatchewan.

2. Unless all the soil under consideration is very uniform, determinations of the hygroscopic coefficient are indispensable. The determination of this value is extremely important even where the soil is uniform.

3. The storage capacity for available water of the two soil types studied, may be placed at from five to seven inches of rainfall for wheat and oat crops.

4. A better idea of the moisture conditions of the soil at Indian Head may be obtained from a casual examination in the field than from the drying and weighing of the samples, unless the hygroscopic coefficient is considered.

5. The moisture stored in the subsoil during the previous summer, and not the frost of the preceding winter, is the cause of the high yields of wheat and oats obtained in southern Saskatchewan.

6. The soil of southern Saskatchewan does not remain permanently frozen at any depth.

7. Investigations of the moisture conditions to a depth of only 12 to 16 inches are of no value and may often be entirely misleading.

THE NITROGEN COMPOUNDS OF THE FUNDAMENTAL ROCKS.

By A. D. HALL and N. H. J. MILLER.

Rothamsted Experimental Station.

It has been shown[1] that many rocks, when samples are taken from such a depth as precludes all possibility of weathering, contain nitrogen in amounts comparable to those present in soil, especially when the strata consist of indurated clays like the Lower Lias, Oxford, Kimmeridge or London Clay. Since the rocks also contain carbon the nitrogen is without doubt of organic origin; the carbon and nitrogen compounds represent the humus present in the clay when it was deposited, and are in fact the mineralised remains of that organic matter. When soil is produced by the weathering of such rocks these carbon and nitrogen compounds will remain wholly or in part in the soil and may there account for a considerable proportion of the total nitrogen they contain. It thus becomes of some importance to ascertain if this nitrogen is ever likely to become available for the plant by the normal processes of bacterial oxidation, or whether it has passed into such a state of combination as to be susceptible of no further change under such conditions as prevail in the soil. If the compounds are too mineralised or bituminised to be attacked by bacteria it would explain the fact that much of the nitrogen present in soils seems to remain permanently beyond the reach of plants. For example the soil of Broadbalk wheat field at Rothamsted, which had been cropped continuously with wheat for 50 years without the addition of any manure, still showed in 1893 very nearly 0·1 per cent. of nitrogen, equivalent to about 2500 lbs. per acre in the top 9 inches of soil; yet the average wheat crop on this plot only contains about 17 lbs. of nitrogen, of which 5 lbs. is supplied annually by the rain, without taking into account any further additions by the action of *Azotobacter* and kindred organisms.

It was therefore decided to subject certain of the deep-seated rocks, the carbon and nitrogen of which had already been determined (*loc. cit.*), to the action of soil bacteria, to ascertain if any measurable amount of nitrates would be produced. A kilogram or so of the rocks was reduced to powder and mixed with sufficient ignited coarse sand to keep them open, and a little potassium phosphate and calcium and magnesium

[1] Miller, *Quart. Journ. Geol. Soc.* 1903, **59**, 133.

sulphates to provide mineral food for the bacteria; when necessary 1 per cent. of calcium carbonate was also added and the dishes containing the mixture were moistened and placed under bell-jars over water and stored. At starting the mixtures were inoculated with the filtered extract from a fertile garden soil, occasionally the mass was stirred and a fresh inoculation given, the nitrogen in the added soil extracts being determined each time. After fourteen months' exposure the nitrates were washed out of the mixtures and determined (Series I). In all cases nitrates were found, in amounts beyond any possible experimental error wherever the rock itself contained an appreciable amount of nitrogen. The trial was repeated on fresh rocks which were again exposed for a year with similar results (Series II). At this stage it was found that some of the rocks contained appreciable amounts both of nitrates and of ammonium salts, which would yield ammonia on distillation with magnesia and which when oxidised would account for more than the nitrates found. Accordingly a fresh series of rock powders was made and distilled in small portions at a time with magnesia and water under reduced pressure in order to get rid of compounds of ammonia. The mass was dried to remove the excess of water, and then inoculated and exposed as before, this time for a period of three months only (Series III). Since the nitrates were not removed with the ammonia before the oxidation began, their amount was determined in fresh samples of the rocks. Again more nitrates were found than were present in the original rock, so that there can be no doubt but that the nitrogen compounds of the fundamental rocks, other than the ammonium compounds, can be slowly attacked by bacteria and converted into nitrates.

The Table gives particulars of the rocks examined—percentages of carbon, total nitrogen, and nitrogen as ammonia and nitrates, also the amount of nitrates formed in each of the three experiments.

The authors conclude that the nitrogen compounds of the soil are not wholly of recent origin, but have in part been derived from the rock out of which the soil has been formed by weathering. In some of the clay soils the proportion of nitrogen due to the original rock is likely to be considerable, which may account for the comparative infertility of many clay soils which by analysis appear to be rich in nitrogen. Such nitrogen compounds are however to some slight extent slowly attacked by bacteria and yield nitrates available to the plant.

The authors are much indebted to Mr H. B. Woodward, F.R.S., of H.M. Geological Survey, for the samples of deep-seated rocks numbered 5, 9, 10, 11, 12, and 13.

Carbon and Nitrogen in unweathered rocks and nitrates produced therefrom.

Number of Soil	Formation	Depth at which Sample was taken in feet	Organic Carbon %	Nitrogen %	C/N ratio	Nitrogen mgs. per kilo.		Nitrates formed mgs. Nitrogen per kilo. after deducting nitrogen in soil extract and nitrates in the rock before exposure		
						as Ammonia	as Nitrate	Series I	Series II	Series III
1	Lower Bagshot Sand, Weybridge ...	18—20	0·02	·00384	5·4	—	—	0·52	—	—
2	Upper Greensand, Farnham, Surrey	30	0·032	·00718	4·5	—	—	0·55	—	—
3	Folkestone Beds, Brabourne, Kent...	20	0·019	·00453	4·2	—	—	−0·2	—	—
4	Lower Greensand, Sevenoaks	30	0·076	·00881	8·7	—	—	2·2	—	—
5	London Clay, London	130	0·391	·041	9·5	9·5	0·49	4·56	—	—
6	Gault Clay, Nackholt, Kent.....	18	0·427	·0415	10·3	14·0	0·69	10·51	—	—
7	Weald Clay, Pluckley, Kent.....	30	0·135	·0647	2·1	19·6	0·42	9·84	—	1·28
8	Carboniferous Shale, Barnsley.....	1236	1·938	·137	14·1	25·2	0·35	9·70	—	1·03
9	Lower Gault, Dover	280—400	0·172	·0325	5·3	—	2·01	—	0·41	—
10	Oxford Clay, Dover	920	0·548	·0528	10·4	—	0·72	—	5·45	1·60
11	Kimmeridge Clay, Welton, Lincs. ...	246	2·139	·107	20·0	—	1·25	—	0·79	0·60
12	Kimmeridge Clay, Dover	570	0·387	·0455	8·5	—	0·39	—	2·78	1·92
13	Lower Lias, Mickelton, Glos.	700	1·120	·0803	13·9	—	1·08	—	1·21	1·84
14	Clay with Flints, Harpenden	10	0·577	·0294	19·6	—	0·61	0·62	—	—
15	,, ,, ,, ...	Surface soil	—	—	—	—	—	8·40	—	—
16	Coal	—	—	1·740	—	—	—	—	−0·5	—

NOTES.

1. A grey coarse sand.
2. Pale grey fine sandy rock.
3. Coarse yellowish sand.
4. Fine yellowish sand.
5. Solid grey clay from the Tube Railway workings below Covent Garden.
6. Solid dark green clay.
7. Close grey and red mottled clay forming a red powder.
8. Hard grey shale from the Wath-on-Dearne pit.
9, 10, 12. Hard grey clays from the coal pit shafts at Dover.
11. Hard grey clay.
13. Hard grey clay
14. Reddish sandy brick earth.
15. Surface soil from the unmanured plot on the Barnfield, first extracted with water to remove nitrates and then sterilised by heating to 110° on two successive days.

MENDELIAN STUDIES OF EGYPTIAN COTTON.

By W. LAWRENCE BALLS, M.A.,
Botanist to the Khedivial Agricultural Society, Cairo.

Introduction.

The Khedivial Agricultural Society of Egypt recently voted a capital expenditure of £2000 towards the establishment of a Mendelian Experiment Station for the study of heredity in cotton.

This Station will form the centre of a system of Seed Stations in various parts of Egypt; the strains of cotton sent out from the Central Station will be tested in these for their agricultural utility, and propagated for distribution to the cultivators.

The project is based on the researches of the last three years; I propose to abstract the results of these researches in the present paper. Various publications in the Year-Books of the Society for 1905 and 1906 give detailed accounts of the preliminary work up to the end of the latter year. The present account will deal only with Genetics, summarising the subject up to date.

Previous Work.

There is not much information of value among the old literature. Some of the work which has been accomplished on the strength and quality of cotton fibre has been of use to me, as have also the accounts of variety-trials. Owing to the absence of any individualistic conception in the published accounts of cross-breeding no evidence of any kind could be obtained from them, even as to the direction of dominance.

The only paper on Mendel's Law in cotton, other than the present writer's preliminary accounts[1], is by Fletcher[2]. Experiments are being conducted by Fyson in Madras, but nothing has been published[3].

[1] This *Journal*, Vol. II. Pt 2; *Khedivial Agricultural Society's Year Book* for 1906.

[2] This *Journal*, Vol. II. Pt 3.

[3] Since going to press an important contribution by H. Martin Leake has appeared in *Jour. and Proc. Asia. Soc. Bengal*, IV. 1. 1908. *Vide inf.* pp. 357, 365, 368.

Initial Problems.

It is widely believed in Egypt that the cotton crop is deteriorating in yield and quality. In the absence of full historical evidence and samples no definite proof of this can be given, but the yield per acre is certainly decreasing. The decreased yield is due to various causes, some of which are outside our province, such as meteorological changes, elevation of the water table, arrest of nitrification through over-watering, insect pests, etc.

In addition to the depreciation of the general crop the cotton experts recognise that each variety deteriorates after it has been grown for a few years. For instance, the original Afifi cotton was composed of strains which matured early in the season, and finished their growth by the time that the modern Afifi is yielding its second picking. During the last two years it has become evident to the grading experts that Yannovitch, the most recent of those varieties which are extensively grown, is beginning to degrade.

Very little was formerly known about the crop from the analytical standpoint; it was even thought possible that the "Hindi" weed cottons might be transmuted into Egyptian plants, and *vice versa*, so that the first necessity before me was to obtain approximate knowledge of the three following subjects:

A. The extent of the fluctuation in the various characteristics of the individual plant, especially of the seed and lint.

B. The number and diversity of the various forms which presumably constituted the commercial varieties.

C. The gametic composition of the individual plants; otherwise expressed, the amount of cross-fertilisation under field conditions. The answer to this problem would be of the utmost importance; its effects would be felt throughout the whole research, in the applied work even more than in the purely scientific portion.

The next stage of the research was to determine the allelomorphs involved in cotton hybrids. Some of these were determined from natural hybrids when investigating the gametic composition of individuals, but the systematic attempt to elucidate Mendel's Law is being made on crosses between cottons differing in many characters, such as Egyptian and American Upland. This method of attack, contrary to the Mendelian principle of investigating simple cases first, was necessitated by the urgency of practical requirements. Nothing has been lost by it, since we are to have facilities for dealing with the large populations which the method requires.

Practical problems arising from the application of the knowledge acquired included the selection of plants from the existing crop, the purification of their splitting-forms; the synthesis of new cot[illegible] earlier maturity, heavier yield, longer, more uniform and better staple, etc.; and the planning of methods for propagating pure strains without contamination[1].

Part I. Analysis of the Crop.

The initial determination of the nature of the crop was made from study of the seed, with its attached lint.

The usual method of examination of cotton seed is to comb the lint out into two wings on either side[2]. This method does not demonstrate a very important character, *i.e.* the relative length of the lint on various parts of the seed. The plan adopted throughout these experiments has been to comb the lint out into a disc, parting the hairs along the raphe.

Measurements were made from the surface of the seed coat to the edge of the disc of hair. This edge is not quite definite, so that a subjective error of a millimetre is involved.

Strength was tested by breaking fibres between the fingers in the usual manner, but it would be well to point out here that strength of fibre is mainly dependent on the suitability of the plant to its environment, though the average breaking strains of various kinds of cotton are by no means the same.

Section 1.

Fluctuation of Seed Characters.

A great deal of description of various kinds of seed cotton has been written, but, as we shall see later, the fact of two cottons bearing the same variety-name is no guarantee of their bearing similar characters. There was no available evidence as to the constancy or otherwise of the various characters, when pure strains were employed.

The following conclusions were drawn in the first instance from comparison of seed formed on the same plants at various stages of growth. The first flowers appear near Cairo in June, and the first bolls are mature in the end of August, while some bolls succeed in opening as late as December. Examination of our meteorological records will show

[1] The writer, *Proc. Cairo Sci. Soc. Cairo Sci. Jour.* No. 17, 1908. With discussion on causes of crop deteriorati[illegible]n Egypt.

[2] *E.g.* H. J. Webber, *Yr. Bk. U. S. Dep. Agr.* 1902, Plate XLIV.

that the first and last bolls develop under very widely different conditions of environment.

Subsequently, the conclusions drawn in this way were confirmed by the study of isolated strains[1], pure in respect of one or more seed characters, which were grown for four consecutive years under varying conditions of cultivation.

Descriptive characters of the ginned seed.

Fuzz, colour. The colour of the short hairs which underlie the cotton lint may be green, brown, or white.

Green fades to brown in sunlight, but some strains retain their colour more firmly than others. White fuzz is not known to me in Egyptian cottons, but in other strains it is quite definite.

Fuzz, distribution. All grades of fuzziness are found, from naked seed with no hairs other than the lint, to seed entirely covered with fuzz. Intermediate types bear a small tuft at the micropylar end only, or a patch at the base of the seed as well.

These four types are quite definite. Some fluctuation occurs in odd seeds, but examination of a sample of twenty seeds will enable one to assign the strain to its type.

Testa, surface. Smooth, or streaked and corrugated.

This fluctuates greatly, even in the same boll. It is useless for comparative examination. Some strains may be permanently streaked, and some permanently smooth[2], but none such have appeared in the fifty plants which were grown from described seeds for this purpose.

Testa, colour. Black, dark brown, olive green, chestnut, or reddish orange.

The last is only found in immature seeds. Black appears to be definite, but the other three are interchangeable.

Seed shape. Long or round, tip pointed or blunt, tip symmetrical axially or bilaterally.

It is an erratic character which seems to be in part determined by the shape of the embryo, and is not yet worked out.

Stalk or Funiculus. Long or short.

A constant character but easily defaced in storage.

[1] *K. A. S. Yr. Bk.* 1906, No. 89.

[2] The character is employed by Watt, however, as descriptive.

Descriptive characters of seed cotton.

Major length of lint. Nearly always at the chalazal end. The occurrence of occasional hairs of abnormal length[1] may be due to irregular environment, and I have not yet seen it in *combed* Egyptian cottons, nor even in cottons notorious for the character[2], if these are grown in Egypt[3].

The length character is one of great importance, and does not seem to have been studied from the individualistic aspect in pre-Mendelian work. These experiments make it certain that every strain of cotton has the property of forming lint of a definite minimum length. Under certain conditions this minimum may be exceeded, but not by 25 %, even under extreme conditions. These extreme conditions are, in Egypt,

(*a*) Cultivation in the cool north of the Delta[4].

(*b*) Crossing with an entirely different type of cotton[5], such as Uplands.

(*c*) Possibly special manurial supplies.

Not by crossing with botanically allied forms, even when such forms come from the other hemisphere and from a very different climate.

When the first bolls on a plant contain lint which is suspected to be longer than the innate length, this real and unintensified length can be determined in two ways, at present:

(i) By comparative examination of the bolls formed late in the year.

(ii) By examination of the few scanty and fragile hairs which are borne on the unfertilised and aborted seeds frequently found in all bolls.

In these situations the lint-length regresses to the normal.

Distribution of lint hairs on the seed coat. Almost uniform all over; denser at chalazal end; micropylar end naked; whole of micropylar half of seed devoid of lint.

[1] F. Fletcher, this *Journal*, Vol. II. Pt 3.

[2] *E.g.* "Griffin" Upland. See *U. S. Dep. Ag. Bur. Pl. Ind.*, "Distrib. of Seed in 1903," p. 7.

[3] H. A. Allard, *U. S. Dep. Ag. Bur. Pl. Ind. Bull.* CXI. 2, explains these abnormally long hairs by simple knotting.

[4] See also *K. A. S. Yr. Bk.* 1906. [5] p. 361.

The fluctuation of this character is slight. It seems not unlikely that this may be in part correlated with the distribution of the fuzz, at least in the case of seeds devoid of fuzz.

Relative length of the lint hairs on various parts of the seed. Most cottons bear shorter hair at the micropylar end than at the chalazal end, but some are quite uniform. The character is one of much practical importance; a strain bearing irregular lint gives an irregular product even if it be pure.

Fluctuation is slight; a lint pattern can be assigned to each strain[1].

Density of lint hairs. The proportion of epidermal cells which develop into hairs is very variable, as would be expected from the cytology[2]. Late bolls bear thinly covered seeds, and unfertilised seed coats may carry as few as a dozen hairs.

Colour of the lint. May be brown, green, or white. The green is found in certain strains of cotton[3] which occur spasmodically among American Uplands, and appears to be identical with the green of the fuzz, which is much commoner. It may have some interest in connexion with the evolution of the cotton plant. These green cottons fade readily in sunlight to brown, and thence to dingy white. When the green colour is transferred to the finer Egyptian lint by crossing, it fades very rapidly, so that in a single day the boll of green cotton is scarcely distinguishable externally from the recessive white. The relation of this phenomenon to the structure of the lint hair wall has yet to be studied.

Brown cottons also fade in the sun, especially Egyptians, though much more slowly than green.

White cotton can always be distinguished from faded coloured cottons.

Strength, twist, lustre, and fluffiness of lint. These fluctuate very much. They are presumably dependent on the amount, uniformity, and texture of the cellulose deposited on the hair cell wall. These characters are dependent on the method of cultivation[4], provided that the plant is suited to the climate in which it is grown; marked initial differences exist of course between various kinds of cotton[5].

[1] Yves, Henry, "Determination de la valeur commerciale des fibres de Coton." Paris, 1902.

[2] *K. A. S. Yr. Bk.* 1905.

[3] "Texas Wool," supplied by D. N. Shoemaker, Esq., Waco, Texas.

[4] Lint born on "rattoons" is always weak.

[5] *E.g.* Sea Island and Short Staple Upland.

Section 2.

Composition of Commercial Varieties.

Once the fluctuation of the seed characters had been determined it was possible to say with certainty whether two cottons were essentially different, or whether they were merely different because of environmental influences.

The examination of the various varieties has been merely a matter of accumulating descriptions which it would be useless to present here. The general conclusions drawn may be stated thus:

1. The Egyptian crop consists of an immense number of different strains (elementary species?) even when seed characters are considered alone.

2. New varieties show no superiority in this respect over the old ones, viz. Ashmouni, Afifi (1883 abt.), Abbassi (1894 abt.), and Yannovitch (1899 abt.).

3. Every character described in the preceding section can be found in the crop with the exception of green lint.

4. The average length of the lint (major length) is about 30 mm., ranging in a symmetrical curve from 18 mm. to 40 mm.

5. Ashmouni bears shorter lint than the other varieties, Sultani (1906 abt.) rather longer lint.

6. The different varieties showed no obvious differentiating characters, other than the colour of the lint. This is readily understood when we consider that this is the only character which can be recognised without subjecting the seed to a searching examination. The quality of the lint is different in certain varieties, thus brown Yannovitch deprived of its colour in the F2 of a cross with white Upland cotton has been taken for "Sea Island, and not Abbassi."

It is only fair to state, however, that the different varieties are said by agriculturists to require slightly different methods of cultivation. Again, cotton experts inform me that Yannovitch is distinguished from Abbassi by a most curious physiological character, *i.e.* that in the former the value of the second picking lint is not much less than that of the first picking, whereas in the latter the degradation in value is considerable. Such differences are none the less averages, and exceptions from the mean are easily found.

SECTION 3.

Gametic Constitution of the Crop.

The experiment on which the first approximate conclusions were formed was conducted in the following way.

Small samples of seed cotton each containing about eight seeds from a single plant were taken at random from commercial samples of several varieties of the Egyptian cotton crop of 1904. Larger quantities would have been advisable, but they were not obtainable at the time. One hundred of such samples were collected, and described; one seed from each was filed for future reference.

The characters employed in the description were four: maximum length of lint, distribution of fuzz on the seed, regularity of length of lint, and distribution of lint on the seed.

These hundred samples were sown in small plots in 1905. Owing to damage done by the "Sore-shin" fungus, which had not then been recognised, only seventy-five sets survived; of these several were reduced to a single plant, but even with this remnant strong indicative results were obtained, which have since been confirmed in other ways.

It should be understood that at the time when the experiment was made we did not even know if the seed characters were purely maternal, though the cytological evidence indicated such to be the case. Neither had we any clue to the course of dominance, nor even any proof of segregation. The analysis of the results of this experiment gave the first data on these subjects, but it will save time in description if we begin at the wrong end, with the following information:

(*a*) The seed characters are purely maternal, excepting possibly the shape of the seed.

(*b*) The allelomorphs involved are:

1.	Long lint.	Short lint.
2.	More fuzz.	Less fuzz.
3.	Regular length of lint.	Irregular length of lint.
4.	Even distribution of lint.	Uneven distribution of lint.

The dominance is complete in each case, the dominant character being written first. The third and fourth pair are less easy to work with, and are only provisionally accepted as yet. There is a greater perceptible range of difference among Egyptian plants in lint length, than in fuzziness, as the following figures show[1].

[1] Table B.

Experimental results. The seventy-five sets of offspring were examined for their seed characters, and compared with the filed seed and description, with the following results:

A.	Offspring all like parent ...	38
	Offspring all unlike parent ...	37
B.	Cases of alteration in individual characters.	
	Maximum length	25
	Amount of fuzz	15
	Regularity of length	16
	Distribution	14
C.	Cases of alteration in groups of characters.	
	In all four	2
	In three	7
	In two	15
	In one	13
D.	Alteration in maximum staple length.	
	Some or all with shorter lint than the parent ...	21
	All with longer lint than the parent	4
		25
E.	Alterations in seed fuzz.	
	Some or all with less fuzz than the parent ...	12
	All with more fuzz than the parent	3
		15

We will assume that all the ovules of a flower are either naturally selfed or naturally cross-fertilised together. This assumption is not quite true, for I have obtained fertilisation artificially by using very small amounts of the foreign pollen, but it is not far removed from the truth according to present evidence.

Formula. Now we know that for y pairs of characters involved in a cross we obtain in F_2

$2y$ homozygotes from $4y$ individuals.

Thus, with our four pairs we get 1 homozygote in 16 individuals, provided that the parent is self-fertilised. Yet, we find that 50 % of our plants were heterozygotes in these four characters. How much cross-fertilisation takes place each year?

Let P and H represent homo- and heterozygote respectively.

In each generation let xP become H and yH become P.

Then the composition of the crop will be as follows:

1st year: P,

2nd year: $(1-x)P + xH$,

3rd year: $\{(1-x)^2 + xy\}P + \{x(2-x-y)\}H$,

4th year: $\{(1-x)^3 + xy(3-2x-y)\}P + x\{3-3(x+y)+(x+y)^2\}H$.

We can now see the law of formation for the H term.

$$\text{In } n \text{ years: } H = \frac{x\{1-(1-\overline{x+y})^{n-1}\}}{x+y}.$$

When "n" is infinite (or practically when more than 10 in the case of our cotton),

$$x\{1-(1-\overline{x+y})^{n-1}\} = x,$$

and

$$H = \frac{x}{x+y}.$$

Example. In our cotton population,

H ... 50 °/₀ or ½,

y ... 1/16.

Hence [1]x ... 1/16 or 6 °/₀.

Thus the amount of cross-fertilisation taking place every year in our crop is about 6 °/₀.

This figure is probably below the truth, on account of the difficulty of recognising alteration in cases where the parents differed but little. We can obtain further evidence from the figures, however, in the following way.

Provided that the annual amount of cross-fertilisation is slight, we can see that the alterations in lint length, for example, will be as follows:

Let L represent long lint, and S represent short lint.

And L is completely dominant over S.

Then, S yields nearly all SS.

Nearly half L yields LL.

Nearly half L yields $LL + 2LS + SS$.

Of the remainder of the population,

When S yields any L or L yields $2LL \div 2SS$	Cross-fertilisation must have happened in the previous year.
When L yields L ...	Crossing may have happened in the previous year.

[1] Misprinted "y" in *K. A. S. Yr. Bk.* 1906.

Since the chances of S crossing with L, and of L crossing with S are equal, therefore

The total amount of crossing of staple-length characters in the previous year (or "x") is got by doubling the number of cases of S yielding L.

Now apply this to the figures obtained experimentally,

(I) Taking the case of lint length only, we have:

4 cases of short yielding long in 75 plants[1].

Therefore, cross-fertilisation in 1904 was 8 %.

(II) Taking the pair, lint length and seed fuzz, there were:

5 cases of recessives yielding dominants in 75 plants.

26 cases of dominants yielding recessives (12 being heterozygotes in both characters).

Thus, $H = 26/75$ or $1/3$ about,

$$y = \frac{2^2}{4^2} \text{ or } \frac{1}{4}.$$

And $$\frac{x}{x+y} = 1/3,$$

$$\therefore x = 1/8,$$

and $$\frac{75}{8} = 9{\cdot}4 \text{ or } 12\tfrac{1}{2}\ \%.$$

Thus by calculating back the probable amount of cross-fertilisation in 1904 from the existing number of heterozygotes, we obtain the result $12\frac{1}{2}$ %.

Whereas, by actual experiment we found 5×2 or $13\frac{1}{3}$ %.

The above figures are too small to be of real value in themselves, but the approximation obtained has since been shown to be a fair one, by notes on the amount of contamination which isolated strains and F1 plants undergo by vicinism.

The method has been described in detail, although the numbers are small because the application of Mendel's Law in some such way should be of use to all students of genetics who are confronted with a demand for the improvement of a crop which is not entirely self-fertilised[2].

It will be noticed that this conclusion—that all kinds of cotton which I have grown in Egypt are liable to *at least* five per cent. of cross-fertilisation per annum—is diametrically opposed to the opinion

[1] Table D.

[2] *E.g.* Sugar, maize and root-crops.

of many writers on cotton. Thus Gammie states his contention to "be that Indian cottons are normally self-fertilised[1]," and quotes Fletcher in support of the view, while Burkill[2] also comes to the conclusion that "insects...in Behar, do produce an effect; but it is an extremely small one indeed—merely a hybrid plant here, and a hybrid plant there," seventeen possible cases in one hundred thousand being the actual figures. It may be that the rule which applies to Egyptian and to Upland cottons does not hold good for the Indian forms[3]. It is also possible that the "variations" due to gametic segregation have not been disentangled from those due to environmental fluctuation; much miscellaneous evidence can be adduced in support of this view, which was certainly held in an extreme form in Egypt[4].

Webber, on the other hand, has shown experimentally that "it would seem that ordinarily only from 5 to 10 per cent. of the seeds are normally cross-fecundated[5]." The objection has been raised to this statement that his results were obtained by study of markedly dissimilar kinds, in order that crossing might be detected easily; this objection is disposed of in the crop analysis described above, for there the seeds were not subject to any isolation nor to experimental treatment, until they were taken from commercial samples to be tested for their gametic composition; the differences between the various strains which were proved to have intercrossed was so slight, moreover, as to be unrecognisable to the agriculturist.

Part II. Mendel's Law in Cotton.

The cotton plant is not an easy subject for heredity experiments; the plants are large, the flowers are not entirely self-fertilised, and many of the characters fluctuate considerably. Numerous pests attack the experimental plot. Two fungi are important; sooty mould in the late summer, and the "Sore-shin" fungus in the spring. The latter attacks the germinating seed, destroying it, or delaying its growth, but the "Naphthalene dressing" is now available for the control of this plague[6].

[1] *Mem. Dept. Ag. India*, II. 2, p. 2.

[2] *Journ. and Proc. Asiatic Soc. Bengal*, New Ser. Vol. III. No. 7, 1907, p. 524.

[3] Leake, *loc. cit.* proves conclusively by study of leaf characters that the speculations about the self-fertilisation of Indian cottons were without foundation.

[4] See p. 347, on the nature of Hindi.

[5] *Yr. Bk. U. S. Dep. Ag.* 1902, p. 370.

[6] *K. A. S. Yr. Bk.* 1906.

The chief insect pests are Aphides, Boll worm, and the cotton Stainer Bug. The two latter are practically uncontrollable.

The rapid response of the growing plant to changes in the limiting factor of the environment makes it advisable to work with statistical descriptions of individuals when such a method is possible; at present this has not been done to any great extent, but with a residential experiment station much more use will be made of statistical methods, in order to study such characters as habit of flowering, time of maturity, etc.

One great advantage of the cotton plant is that it can be grown on as a perennial by simply cutting it back and allowing it to shoot from the base in the following year; thus, if more seed is required, or protected seed in place of natural seed, it can readily be obtained year after year.

There are three points of the utmost importance to be borne in mind when experimenting on genetics with cotton. These three points belong by right to the conclusion of this paper, since they are deductions from the results described in it, but it is well to recognise their importance at the outset.

These points are:

1. No pedigree can be considered exact, unless the direct ancestors have been all self-fertilised. Even five per cent. cross-fertilisation per annum will entirely vitiate the statistical expressions of Mendel's Law in the F3. When, as in pre-Mendelian times, selection was carried on in mass-cultures for as many as seven years, the chances of a plant representing its name at the end of that time were probably even! Absolute freedom from vicinism is perhaps impossible on a large scale, but the use of netting[1] abolishes it when netting is feasible.

2. The desirable characters are mostly dominant over the undesirable ones[2], except in cases where the heterozygote is intermediate. This makes deliberate synthesis of commercially useful cottons more tedious than it might otherwise be.

3. It must be remembered that the parent plants used in a cross are almost certain to be heterozygotes in some characters, for the conclusions drawn in the first section of this paper appear to apply equally to all the kinds of cotton which I have yet examined. Consequently, the descendants of each F1 plant should be kept separate from those of the others.

[1] p. 359. [2] p. 362.

The point might seem obvious, but it does not appear to have been fully realised by some workers who are dealing with hybrid crops.

4. One is tempted to add that the intensification by crossing of various characters (such as lint length[1]) is likely to give rise to unfounded expectations on the part of those who are doing synthetical work, but there is some evidence from other organisms to show that this intensification may perhaps be permanent.

Methods. The keeping of records of all kinds, taken at all stages of growth, and all hours of the day, in addition to laboratory examination of the seed, is necessitating the development of special card-filing systems in preference to the use of note-books.

Seeds which have been combed out for examination are also stored for reference in card-files, by covering them with squares of celluloid, the corners of which are inserted in slots in the black cards. An identification label is provided, and descriptions can be written on the white back of the card.

Stout paper bags are employed for storing small samples of lint and seed cotton. These are filed vertically with guide cards in sets of drawers made on the "elastic" system, so that the drawers can be taken into the field and filled with the samples directly. Much sorting of samples is obviated by this plan.

The most important details of method are those dealing with the exclusion of bees by nets, and the prevention of "Sore-shin." The latter has been referred to elsewhere, and it is only necessary to point out the immense importance of such prevention, in that it enables us to raise many more seedlings from a limited quantity of seed.

The netting of plants is at present done with mosquito nets, each two metres high and one metre square, the top being made of stout calico. These nets are supported on two-inch-square sticks, which are placed inside the net, one at each corner. The upper end of each stick is covered with a rough pad of rags to prevent chafing of the calico; the lower ends are surrounded at the ground level by four thin boards, measuring 100 cm. by 30 cm., placed on edge, and the foot of the net is rolled up and held against these boards by an encircling cord. Unless these foot-boards are used the net rots at the soil level. We estimate that with care, and occasional spraying, these nets should last three years apiece. They cost about ten shillings each, but they are as

[1] p. 361, etc.

essential to the conduct of the experiments as the cotton plants themselves.

For propagation of pure strains on the Central Station it will probably be found cheaper to build large cages of fine wire net, each holding some fifty plants. The seed from these will be sent out to the sub-stations for propagation.

The cross-pollination of the cotton flower appears to be effected almost entirely by bees. Many insects are to be found in the flowers, and a list is being prepared by Mr Willcocks[1], but all those which are capable of bearing foreign pollen[2] are excluded by the nets; there does not seem to be any appreciable amount of wind-fertilisation, and even wind-borne pollen would probably be arrested by them.

Before the nets were adopted the preparation of self-fertilised seed was laborious and uncertain. Tissue paper bags were employed for separate flowers, and a large proportion of the bagged flowers was shed afterwards, probably on account of interference with transpiration.

The operation of crossing has also been simplified by the nets. The flowers are castrated at 4 a.m., and cross-pollinated at 9 a.m. No bags are necessary if the other open flowers under the net are removed. The flower from the plant which is to be employed as the male parent is, of course, covered with a bag of paper.

The First Generation.

The details concerning dominance will be described under the next heading.

The crosses on which the results were obtained were as follows: the common names alone are given, which will enable those acquainted with cotton to recognise the types dealt with, while details as to characters will be gathered from the second generation descriptions. It seems somewhat useless to attempt to give rigid names to hybrid plants, such as the Afifi parent in 252.

Crosses in Second Generation, 1907.

250. Texas Wool (Upland) × Abbassi (Egyptian).

251. Afifi (Egyptian) × Natural hybrid of Hindi and Egyptian.

252. Afifi (taken from Brown Yannovitch) × Truitt Big Boll (Upland).

[1] Entomologist to the *K. A. S.*

[2] See also Burkill, *loc. cit.*; H. Müller, *The Fertilization of Flowers*, p. 145; W. Trelease, *Nectar*, Washington, 1879; Toniet, *Histoire des Drogues*, Paris, 1694, Plate of Cotton plant with insects flying.

Cross in First Generation, 1907.

253. Hindi (Egyptian weed cotton) × Charara (Egyptian).

254. Charara × Wild cotton of Moqui Indians from Arizona.

In no case were all the plants of the F1 identical. The existence of heterozygotes in one or both parents was shown in the following characters, by one or another.

Seed fuzz, length of lint, spot of leaf, shape of leaf, habit of growth, colour of corolla, colour of anthers, and seasonal formation of flowers.

The most striking feature is the intensification of certain characters which results when two botanically dissimilar cottons are crossed together. This intensification is shown in the height of the plant, the time of flowering[1], the length of the lint, the size of the seed, probably in the fuzziness of the seed, etc. Of these the length of the lint has been observed most fully.

In cross 252 a 30 mm. cotton was crossed with a 20 mm. cotton. The 30 mm. one was known to be heterozygous in length of lint, because its offspring had comprised both 30 mm. and 24 mm. cottons. We should have expected that the F1 would consist of equal numbers of 30 mm. and 24 mm. cottons, both being heterozygotes containing the recessive 20 mm. form.

Such was not the case, for when the three F1 plants ripened, two were found to bear 30 mm. lint, while the lint of the other was 35 mm. long. By comparison of this series with another one (250), where the parent's lint had been respectively 25 mm. and 23 mm. long, while the F1 bore 30 mm. lint, it was clear that the two allelomorphs of the heterozygote parent in the former series had been separately acted on by their union with the American 20 mm. gametes in such manner as to stimulate them; the result of this stimulation (or possibly reconstruction) being that the character which they controlled was hypertrophied.

That the character is essentially unchanged may be seen by examination of the lint born in the late bolls at the beginning of the winter, or else of the few lint hairs born on those unfertilised seeds which are found in all bolls. In these two situations the major length of the lint is found to be the same as that of the parent; the 35 mm. F1 drops to 30 mm. and the 30 mm. F1 drops to 24 mm. This shows that the intensification is connected with nutrition; not with temperature, except in so far as the temperature may limit the food-supply. This

[1] In cross 254:—1st flower in parent-strains, and F1, appeared on following dates: *Hindi*, 1st three plants, June 11th, 15th, 30th; *Charara*, 1st four plants, June 12th, 13th, 14th, 14th; F1, three plants, June 4th, 5th, 6th.

conclusion is supported by the fact that the same intensification in a slighter degree can be effected on the Egyptian parent by growing it in the north of Egypt.

In contrast to this we have the series 253 in which practically no intensification of length was found in F1.

This intensification on crossing is a well-known phenomenon. Many of the old examples are not cases of intensification proper, but omitting these we have still a number of cases which are parallel to ours. The question now arises as to the stability of such intensified characters in subsequent generations. Some results obtained by Biffen[1] on intensification of the length of the internodes of wheat ears indicate that the intensification may be permanent in some strains[2]. If this is so for cotton we may hope, contrary to my first expectation, not merely to combine existing characters into one strain, but even to make new characters. In any case, this is by far the most interesting and important problem offered for our study by the cotton plant.

The Second Generation.

The evidence at present available is based on naturally fertilised seed, with a probable error of 10 %. The result of this is that hardly any of the existing F3 embryos will be grown, and next year will be devoted to a thorough examination of four large sets of F2 plants grown from netted plants. Some experience has been obtained this year in the methods of obtaining and treating statistics for the individual, and these methods will be applied on a large scale.

Provisional List of Allelomorphic Pairs.

Complete dominance.

*Glabrous petiole in seedling.	Hirsute petiole.
Light green leaf.	Dark green leaf.
Long filaments of anthers.	Short filaments.
Long style (?).	Short style (?).
Large seed.	Small seed.
*More fuzz on seed.	Less fuzz.
*Coloured fuzz.	White fuzz.
*Long lint.	Short lint.
Regular length of lint.	Irregular length.
Even distribution of lint.	Uneven distribution.
*Egyptian quality of lint.	Upland quality.

[1] Unpublished.

[2] See however, Johanssen, "Int. Conf. on Plant Genetics," *R. Hort. Soc. Jour.* 1907.

Heterozygote intermediate.

*Red spot of leaf.	Faint spot.
*Yellow petal.	Cream (white) petal.
*Large purple spot on petal.	No spot.
*Rich yellow anthers.	Buff anthers.
First stem-branches flowering.	Branches vegetative.
Tall stem.	Short stem.
Early flowering.	Late flowering.
Early cessation of flowering.	Continuous flowers till December.
Early maturity.	Late maturity.
Coloured lint.	White lint.

* Asterisks denote reliable evidence.

This list is compiled from two sources. Firstly, the pedigrees of some of the natural hybrids referred to in the first section of this paper; secondly, from the examination of the F2 of the following:

252 A ... ... 56 plants.	252 B ... ... 30 plants.
252 C ... ... 30 plants.	250 A—G ... 75 plants.

The comparison of the offspring of the three sister plants, 252 A, B, and C, has enabled many points to be seen which must otherwise have remained obscure; because, on account of the unpleasant conditions under which the work has been carried on up to the present, it has not been possible to obtain sufficient data about the parents which were employed.

The characters will be discussed in the order in which they appear during the growth of the plant. Numbers obtained are aggregated when all the sets are comparable; plants stunted by "Sore-shin" are omitted when dealing with physiological characters; hence the irregular totals.

Red spot of leaf. The character is due to the development of anthocyan in the epidermal and sub-epidermal cells of the petiole at the point where it divides into the leaf-veins. The colouration of the "red-leaf" Upland cottons[1] is due to further development of the same character.

Like all anthocyan colours it is dependent on the illumination, and also on temperature, and water supply. Under similar conditions of cultivation the types are sharply distinguished.

Crosses of spotted with spotless give spotted F1, but the intensity of the colour is less than in the spotted parent. The heterozygote spot

[1] *E.g.* "Willett's Red Leaf."

appears to be distinguishable from the homozygote spot in F2. The following count was obtained on all seedlings:

Spot (homo- and heterozygote)	... 187
No spot	... 65

The character may be regarded as a capability of reaction to *light*. It is also of practical importance in connexion with the eradication of the weed-cotton called "Hindi" from the Egyptian crop, since the former has a full red spot on the leaf, whereas the great majority of the Egyptian plants have only a faint spot. The F1 of natural hybrids between Hindi and Egyptian can be detected better by this character than by any other.

Hairiness. The petiole of the Egyptian parents bears a few hairs on the dorsal side, and a very few sparse hairs on the ventral side; the petiole of the Upland parents is hirsute all round, though more densely so on the dorsal side.

Petiole of the F1 seedling resembles the glabrous parent.

The following count in F2 was obtained:

Glabrous	... 111	Hirsute	... 37

This expression of the case does not appear to cover the whole ground, for the hairiness of the leaf surface and stem appears to be separate factors, possibly correlated with the simple one described.

Leaves. The leaf-characters are the hardest of all to judge, on account of the many factors which appear to make up the leaf shape; a good deal of fluctuation has also to be eliminated in the early stages of the plant's growth[1], and some plants appear to form mature leaves at an earlier stage than others. They will be more easily studied in the F3.

The form of the leaf would not appear at first sight to be of much practical importance, but the sensitiveness of the "lint-quality" is such that the shape and size of the leaves may be able to affect it.

In the series 252 the Upland parent had a lighter green leaf than the Egyptian. F1 was intermediate, and dark green plants were recognisable in F2:

Light green	... 93	Dark green	... 28

[1] T. H. Middleton, *Agric. Ledger*, 1896.

In the matter of other leaf characters the following analysis seems to be indicated[1]:

(*a*)	Large lamina.	Intermediate lamina.	Small lamina.
(*b*)	Slightly dissected.	Deeply dissected.	Very deeply dissected.
(*c*)	Broad segments.	Medium segments.	Narrow segments.

(*d*) Shape of tip of segments.

(*e*) Flatness of lamina.

(*f*) Thickness of leaf.

(*g*) Hirsuteness.

Height of stem. The height of the plant is intensified in F1 to a considerable extent, perhaps 50 %. As we do not know to what this intensification is due we should hardly expect to find conspicuous segregation in F2.

Curves of height were plotted for both series 250 and 252 at the end of June and again at the end of July. The results show very little, but indicate that segregation has occurred, together with intensification.

Series 250. Both parents of about the same height, *e.g.* on a given date about 60 cm.

F1 on same date would have been perhaps 100 cm. high.

F2 curve of heights on same date ranged from 30 cm. to 100 cm., although the plants had been practically level till two months old.

Series 252. The Egyptian parent was very tall, and bred pure to the character of tallness through three generations, being one of the tall plants which are common in the Egyptian cotton fields, and are by some supposed to represent the remains of the old variety "Hamouli."

The F1 plants were all taller still, when growing side by side with the parents in 1906.

F2 curve of heights again showed a range which exceeded that of either parent, with indications of three crests, corresponding to 1 : 2 : 1.

The subject will be investigated next year by taking measurements every fortnight, through a large population. The growth-time curve of the individuals is likely to show important results.

Habit of growth. The form of the stem is subject to some fluctuation. There seems to be at least one character concerned which cannot be obliterated, and provides an allelomorphic pair.

[1] See however, Leake, *loc. cit.*, who has given us a statistical expression for some of these characters.

The distinction within this pair lies in the nature of the first branches produced by the seedling plant; these may be monopodial and vegetative, or sympodial and flowering directly. In the former case the stem branches at the base, while in the latter the stem grows straight up, putting out only flowering branches. There also seems to be a second character which may be superimposed on this, namely, whether basal branches are put out late in the summer or not, as we shall see shortly.

We shall refer to the form in which flowering branches alone are formed at first as "unbranched" from the vegetative point of view, and to the other as "branched."

The cross of these two types (of which the latter is the usual one among Egyptians) gives a kind of intermediate in F1, in that it sends up a leading shoot with flowering branches, but quickly surrounds this at the base with laterals.

In the F2 we had the following figures in the middle of June:

250 A, G.	Branched	... 58	252 A.	Branched	... 21
	Unbranched	... 22		Unbranched	... 27
252 B.	Branched	... 18	252 C.	Branched	... 18
	Unbranched	... 8		Unbranched	... 6

On this date, it will be noticed, while 250, 252 B, and 252 C gave a plain 3 : 1 ratio, the ratio in 252 A was 7 : 9. This might be taken as an indication (and nothing more) of the existence of a second pair of allelomorphs interacting on the first pair. Further probability is given to this idea by a later count made at the end of July, when the so-called "second growth" has begun in the field crop.

The result of this second count was:

250 A, G.	All branched.	252 A.	Branched ... 35
252 B.	Do.		Unbranched ... 13
252 C.	Do.		

Now we know that the Egyptian parent in 252 was a hybrid in respect of length of lint, and we shall see shortly that the curves of flower-production are entirely different in the 252 A series from those in the series 252 B and C, so it is by no means unlikely that it was also hybrid in respect of habit, although pure tall. The Upland parent gave uniform "unbranched" descendants.

There would seem to be a cryptomere present, but it would appear that the "cryptomere" is only such in the early stages of growth. However rash this speculation may appear, it is certain that the differences between these series were not merely accidental.

Flower colour. The flower colour characters have shown a pleasant simplicity by comparison with the preceding characters, and have not yet fulfilled my hopes of complexity[1].

The units involved and the heterozygote forms are:

Homozygote.	*Heterozygote.*	*Homozygote.*
Yellow petal.	Lemon petal.	Cream (white) petal.
Large purple spot.	Small purple spot.	No spot.
Rich yellow anthers.	Pale yellow anthers.	Buff anthers.

Fletcher[2] has shown also that entirely red petal is dominant over yellow petal, which agrees with the behaviour of the partially red (spotted) petal. His red parent seems to have been a heterozygote containing yellow.

These colours are all due to the sap, plastids being devoid of colour.

Series 250. The Egyptian parent had a very small spot, which was nearly extinguished by fluctuation in some flowers. The character was presumably pure, giving no other form in the ten descendants which were grown. The excess of spotless forms shown in the F2 is certainly due to increased fluctuation, for it was common to record a plant once or twice as spotless, and then find a flower appearing which bore a faint spot on one or more petals. The same was true of the F1. The counts in F2 gave the following figures, each of the seven strains showing all three forms:

Petal: Yellow	... 16	Lemon	... 36	White	... 17
Spot: Spot full	... 5	Spot faint	... 9	No spot	... 47
Anthers: Yellow or pale yellow	...	... 47	Buff	... 22	

Series 252. Here the Egyptian parent had a full and conspicuous purple spot, which could not be extinguished by fluctuation.

Petal: Yellow	... 21	Lemon	... 50	White	... 23
Spot: Spot full	... 23	Spot slight	... 42	No spot	... 31
Anthers: Yellow or pale yellow	...	... 72	Buff	... 22	

A partial count of the yellow to pale yellow anthers gave 13:28. These new colour combinations are very handsome in some cases, and might be turned to practical account in providing identification marks for new strains of cotton. One point worth further attention was noted in working out the ratios of the various combinations of colours; it might be due to vicinism, or to partial gametic coupling, but instead of getting the expected ratios of the various combinations we find

[1] This *Journal*, *loc. cit.* [2] *loc. cit.*

an excess of what we may call "positive colouration" at one end of the scale, and of "negative colouration" at the other. The matter will receive attention next year, and the preliminary facts given here may be of interest to other workers.

In series 252 there were 94 plants. The expectation on 96 plants is placed in brackets after each figure.

	Full spot.	Spot slight.	No spot.	Total.
Yellow petal ...	11 (6)	5 (12)	2 (6)	18 (24)
Lemon petal ...	9 (12)	27 (24)	17 (12)	53 (48)
White petal ...	3 (6)	8 (12)	12 (6)	23 (24)
	Yellow petal.	Lemon petal.	White petal.	Total.
Yellow anthers...	17 (18)	43 (36)	12 (18)	72 (72)
Buff anthers ...	1 (6)	10 (12)	11 (16)	22 (24)
	Spot full.	Spot slight.	No spot.	Total.
Yellow anthers...	23 (18)	31 (36)	18 (18)	72 (72)
Buff anthers ...	0 (6)	9 (12)	13 (6)	22 (24)

My expectation that the colour inheritance might be complex was based on the fact that these colours are all closely related substances, thus, the spot character might have been manifested only when the morphological and chemical factors required for its formation had met in zygote formation. This is not the case, but these figures seem to indicate possible partial coupling.

With regard to the occurrence of 2·5 % of red flowers among the descendants of yellow recessives, described by Fletcher[1], it would seem very probable that this was due simply to vicinism. There seems to be no reason why vicinism should be of less importance in India than in Egypt[2], and the percentage obtained by Fletcher comes well within the Egyptian average, even if the number of red flowers in the vicinity was much less than the number of yellow flowers.

Other flower characters. Counts have been made of the length of the filaments, column, and style. The two latter characters fluctuate too much to be distinguished, unless statistical methods can be used. Long filaments appear to segregate from short, the former being dominant.

These characters are worth attention in one respect: the combination of long filaments and column with short style should be a very difficult flower for any insect to cross-fertilise. It might be possible, perhaps, to synthesise in this way a strain of cotton which would be practically cleistogamic.

[1] *loc. cit.* [2] Confirmed by Leake, *loc. cit.*

Tubular corollas appear to segregate from the campanulate corolla found in Upland cottons.

Calyx characters also fluctuate considerably, and will have to be followed out in later generations.

Flower formation. The immensely important question of the time of maturity is connected with this character.

In studying flower formation all plants which have been stunted by "Sore-shin" are disregarded, such plants having been noted in the seedling stage. Habit of growth has also been taken into account, but the only difference which it appears to make in this respect is that the "unbranched" plants are a few days earlier than the rest on the average; this is seen when the curve of the date of the first flower is plotted separately for both habits.

The plot was examined every day from June 3rd, 1907, to the present date (December 31st), and a note made every day of the plants which were in flower. The data thus obtained have been plotted into curves for the individual plants, as well as into other curves, and the results are so interesting that the method will be applied to at least a thousand plants next year, in spite of the labour it involves.

Miscellaneous observations on the behaviour of foreign cottons in Egypt, and of Egyptian cottons elsewhere, have given a strong probability to the idea that each strain of cotton has a definite relationship to temperature in regard to flower-production. Such observations are familiar to all who have grown foreign cottons (if taking precautions to exclude hybridisation); supposing such relationship to exist, it can be more readily seen in Egypt than in any other cotton country, on account of the regular way in which the weather changes as the year goes on. In series 252 the Upland parent was, as usual, earlier in flowering and maturity than the Egyptian.

The results obtained were first plotted to show the date of the first flower, of the average of the first two, and of the average of the first three. The result was practically the same, which ever method was employed, and the first was finally adopted. The curve for the 252 A series appears to indicate that the range is beyond that of either parent, as in the case of height, and the grouping of the dates shows at least an indication of 1 : 2 : 1 ratio.

The curves for 252 B and C were quite similar in form, but the late portion in 252 B was moved up about ten days later. This looks like another character in which the one parent was heterozygote.

The "pure lateness" or "pure earliness" of the two extremes of the curve can only be settled in F3. Meanwhile we have some more results of no little interest.

When the total number of flowers produced on each day of the year was averaged in ten-day periods, plotted separately for 252 A, B, and C, and aggregated for 250 A...G, the curves given in Fig. 1 were obtained. It will be seen that 250 and 252 A give a double-crested curve which is quite distinct from the single crest given by 252 B and C. To ascertain the meaning of this difference we have to determine the form of the curve produced by the individual plants.

Seasonal Production of Flowers.

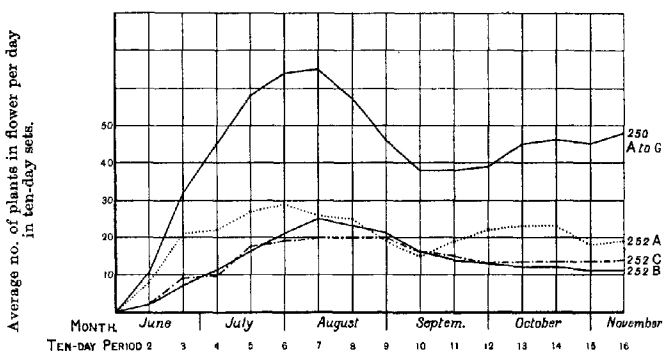


Fig. 1.

When the curve of flower-production in time is plotted for each plant we find some remarkable differences. The general rule which governs these differences has not yet been seen, and it would take too much paper to reproduce every curve. Four types exist:

I. Flowers cease in September. A few appear in December.

II. Flowers cease in September. Flowering resumed in October.

III. Flowers decrease in September and increase again till December.

IV. Flowers continue steadily through December.

The types II. and III. are less common in 252 B and C than in 252 A.

Comparison with the records of other characters shows no connexion of these types with either habit or morphological characters, or even with the date of appearance of the first flowers. The only character common

to any class is that the plants included in I. were marked off in the beginning of September as "early death"; at that time they were rapidly maturing all their bolls and seemed to have ceased work for the year. For the past three years it has been noticed that Uplands, Hindi, etc., start a kind of second growth in the winter, and bear abundant flower buds, but this analysis of an F2 has given the character a definite expression. The character is one of no small importance from the practical point of view, and will have to be worked out with the assistance of a cotton expert to determine the effects on the lint[1].

There are also great differences among the various plants in respect of the number of days on which flowers are produced; some flower very freely, producing many flowers every day, like the F1; while others only flower occasionally. This does not seem to affect the form of the curves of flowering, but only their height. Both slow and rapid flowering are found in all four classes.

Boll characters. These have not yet been investigated in detail. The shape, size, number of divisions, and the appearance of the glands seem to be the four main characters. They will be worked out more easily on the cross of Russell Upland with Charara, since the bolls in the former are very large.

Weight of first picking in 252 series. All seed cotton ripe on August 12th was picked off and stored. A second picking was taken on September 7th. These pickings were weighed separately for each plant, and the results show some trace of regularity.

The only correlation which could be found between the figures obtained and the other data concerning such points as the flowering curve, date of first flower, length of lint, height of plant, etc., was that all plants which produced more than ten grams of seed cotton by August 12th belonged to the group which bore the first flower before June 20th, while those which had not produced any seed cotton by September 7th belonged to the group which produced no flowers until after June 30th. The date of production of the first flower may thus be used as an index to the time of maturity.

It is singular that the curve of weight of the pickings shows a small node in 252 A, B, and C also. This node is composed of a small number of plants which bear distinctly more than the rest, and its appearance in all three sets leads one to think that its presence may not be due to mere accident[2].

[1] See p. 375.

[2] Possibly due to conjunction of large boll.

It is also of interest to note that three plants of the five which were known to be vicinists gave figures for weight of seed cotton which were entirely removed from the rest.

Seed Characters.

Weight of seed. The Upland parent in series 252 had a much larger seed than the Egyptian parent. The seed of the F1 was heavier than either parent, and in F2 the curve reproduced here was obtained (Fig. 2). The weights were determined by counting the number of seeds in five grams of ginned seed from the first picking. Small seed segregates from large seed, and the segregated types show a greater range of fluctuation than is shown by either parent.

Weight of Seed.

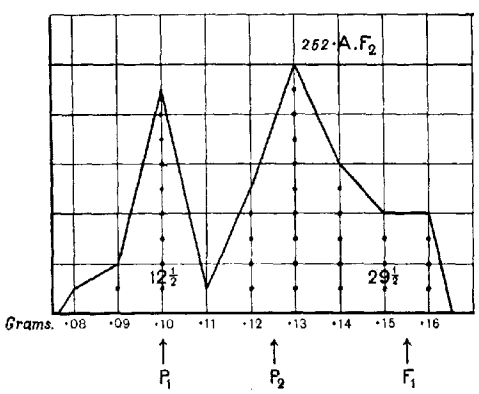


FIG. 2.

Fuzz of seed. The inheritance of this character in cases where the two parents both bore small amounts of fuzz has been followed out in several natural hybrids, and appears to be quite simple, the type with more fuzz being dominant over the type with less fuzz.

In the series 250 and 252 the matter appears to be complicated, but the fact that the F1 was naturally fertilised prevents a definite statement. The slight fuzz of the Egyptian parent was completely dominated in F1 by the entire fuzz of the Upland parent. The slightly fuzzy seed reappeared in F2, but in very small proportions, only 6 in 103 plants of

252 F2; eight others were "semi-fuzzy." Whether this implies the existence of two allelomorphic pairs[1] in crosses where entire fuzz is involved (as is probable) or whether it is due to vicinism, is a matter of considerable importance in constructive work.

It is of interest to note that in the 250 series the Egyptian parent was a natural hybrid made in 1904 between a plant with naked seed and a plant with slight fuzz on the seed; seven F1 plants were raised from a cross of this heterozygote with Upland bearing entire fuzz, and a small F2 raised from each of them. In four the slightly fuzzy type split out, while in the remaining three the naked type recurred; the expectation being of course two to two. The history of this slightly fuzzy strain, derived in 1904 from an unknown pollen parent, dominating the naked seed in 1905, throwing pure recessive naked in 1906, hidden under the entire fuzz of the Upland in 250 F1, and reappearing as a recessive in its turn in 1907, is an excellent example of the grip which the segregation law gives us on the problems of cotton breeding.

Colour of fuzz. The colour is independent of the amount of fuzz. Green fuzz is incompletely dominant over white and brown, but as the latter can be derived from the fading of green, the character will have to be worked out with more suitable material, probably by the use of seed dissected from unripe bolls[2].

Colour of lint. Brown lint (or green) is not completely dominant over paler or white lint, but gives an intermediate heterozygote form. The series 252 A, *e.g.*, gave in F2 the following figures:

Brown	... 12	Cream	... 21	White	... 11

The distinctions in colour cannot be seen well in seed cotton, unless—as in this series—the difference is very marked. This accounts for the conclusion formerly stated that dominance was complete[3]. It is needless to say that the power to differentiate the homozygote without growing its offspring will save much trouble in constructive work.

Length of lint. Long lint is completely dominant over short lint, with intensification of the dominant character in some cases. In F2 the recessive short reappears, and intensification survives partially.

The difference between the parents in series 252 is not sufficient to differentiate the two forms of long and short, since the intensified short

[1] Series 253 gave "reversion" to entirely fuzzy seed in F1!

[2] I have since found by accident that the fuzzy colours are quite distinct when the seed is inspected in bulk from about five metres away.

[3] *K. A. S. Yr. Bk.* 1906.

may be as long as the unintensified long; the curve of lint length given for 252 A F2 (Fig. 3) shows that many of the plants have regressed to the normal, and the question arises as to whether this will continue to happen in successive generations, or whether some strains will retain the intensified length. The long and short forms can be distinguished in the overlap of the curves by comparative examination of the lint formed in the latter part of the growing season, but the methed is tedious; mere examination of the very last bolls is not sufficient, for with some plants the intensification reappears in December.

Length of Lint.

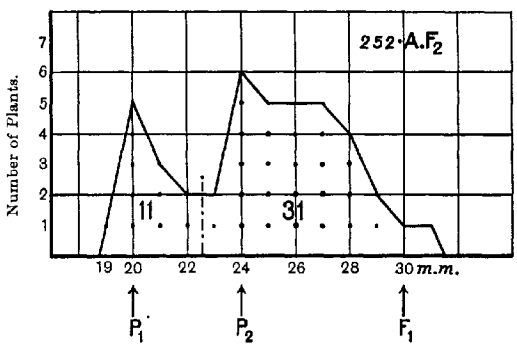


FIG. 3.

There is a possibility that this intensification of length can only be manifested by lint of Egyptian quality (as distinct from Upland). The lengths of the Upland qualities which segregated out in this F2 (see "Quality of Lint") were as follows:

20 mm....3 21 mm....2 23 mm....2 24 mm....2 25 mm....1

These divergences from 20 mm. and 24 mm. are within the error of measurement, and contrast strongly with the fluctuation of all the Egyptian qualities of this generation.

Regularity and uniformity of the lint. The irregular character reappears in F2, but it would be better to wait for the F3 before making a definite statement, as it is not an easy character to judge fairly when the length of lint, fuzz of seed, size of seed, etc., are mixed up with it; the same difficulty holds in examination for uniformity of distribution.

Quality of Lint.

Description of samples of lint from Series 252, *by E. A. Benachi, Esq.*

Parents

45 A. American quality. White short staple, not equal to any Egyptian cotton.

77 A1. Brown in colour, long staple, Afifi style. Not finer than Tanta Afifi. Medium strength.

77 A2. Afifi.

77 B2. Afifi style and colour.

First generation.

252 A. Similar to full quality and colour of Yannovitch[1].

Second generation (252 A1, etc.).

1. Yannovitch colour but short staple.
3. Light creamy colour, poor staple and weak.
4. Light creamy colour, Yannovitch staple and finish, but not so long.
5. Creamy Yannovitch colour, but staple of poor Afifi.
6. Light colour like Yannovitch, but quality like fine staple of Afifi, and not strong.
9. White, but not dead white. Finer than Hindi. Not desirable cotton.
10. Afifi colour, poor staple, short.
11. White, inferior to Abbassi.
12. Good dark brown colour, but very short staple, not Egyptian.
13. Good brown colour, good staple and strong. (Good all round cotton, like the early Afifi of years ago.)
14. Afifi colour, but staple more short and irregular than any Egyptian cotton, though fineish.
15. White cotton, Abbassi colour but not as long. Finer staple than Abbassi.
16. White, but not dead white colour. Short staple, undesirable cotton like the Hindi.
17. Creamy. Inferior to Yannovitch in staple and fineness.
18. Rather light brown. Good quality of Afifi, regular and good staple.
19. Afifi brown colour. Good staple, regular lint and strong, like good style of Afifi.
20. Creamy but short staple, American style.
21. White, not equal to good Egyptian Afifi, shorter staple.

[1] *I.e.* Creamy, finer and more silky than Afifi.

22. Light creamy colour, but staple like Afifi.
23. Creamy colour like Yannovitch, and finer but not strong staple.
24. White, finer than Abbassi, good staple.
25. Light cream Yannovitch colour, long staple and strong. Better than Yannovitch quality.
26. Good brown colour Afifi, good staple and regular, but not fine enough.
27. Fine Yannovitch staple and colour, strong but not very long. Consider it an improvement on Yannovitch quality.
29. Creamy, inferior to Yannovitch in staple and fineness.
30. Light creamy colour, short, American style.
31. Good brown colour, but not Egyptian quality.
34. Whitish, inferior to Abbassi.
37. White, finer than Abbassi, not up in staple or strength.
38. Creamy, but staple short, inferior to Afifi.
39. Creamy colour like Yannovitch, but finer staple and shorter than Yannovitch.
40. White, inferior to Abbassi.
41. Creamy colour, staple short, not like Egyptian.
42. Creamy, short but fineish. American style.
44. Creamy. Yannovitch style. Good staple, not strong.
43. Brown colour, short and wasty. Staple weak.
45. Creamy, but like short staple Afifi.
46. Creamy, very good staple and strong. Better than Yannovitch. (Strongly recommended.)
48. White, but short staple, inferior to Abbassi.
50. Brown colour, good long staple and fine. Fairly strong. (Good cotton.)
51. White colour, strong and fine silky staple, not very long.
52. Abbassi white, not as long, but finer and strong staple.
53. Brown, but Upland staple.
54. Creamy, but short. Inferior to Yannovitch.

The above criticisms were written by Mr Benachi on the hand-ginned samples of first picking lint from the F2 of 252 A. The only special request made was that as colour would be no criterion of the nature of the cotton it would be advisable to denote the quality both with and without the colour.

Perhaps the most remarkable point about the whole list is the comment on No. 13. This plant is not at all like an Egyptian in

appearance, for it has a large dark green leaf, white flower with heterozygote purple spot, heterozygote stamens, flowered on June 11th, and so forth; it has a curve of flower formation which belongs to Class I., and this was apparently the type of flowering curve of the original Afifi strain; by simple examination of the lint from an unknown plant, Mr Benachi has detected this "early death" character!

Tabulation of this list gives the following figures:

(A) White ... 11 (11) Cream ... 21 (22) Brown ... 12 (11)

(B) Other than Egyptian... 10 (11) Egyptians ... 30 (33)

Not defined ... 4

It is thus clear that we may use American Upland parents in synthetical work, for their desirable qualities, and get rid of the undesirable recessive lint character in the F2.

As regards the classification of the Egyptian qualities inside the group, the figures are as follows:

Afifi...	... 7	Finer than Afifi...	... 1
Yannovitch...	2	„ „ Yannovitch...	5
		„ „ Abbassi	... 7
	Inferior to Afifi ...		... 4
	„ „ Yannovitch		... 5
	„ „ Abbassi		... 2

The probable explanation of these classes is that intensification takes place in the silkiness of the lint as in other characters, so that an extra silky Afifi—as in the F1—is equal to Yannovitch. The matter requires much more investigation, but it would seem to be fairly simple. This "mock Yannovitch" will probably provide a companion example to the "unfixable" Andalusian fowl.

In conclusion I wish to express my sincere thanks to my chief, Mr G. P. Foaden, for his advice and information on agricultural and commercial matters relating to Egyptian cotton, and also for the support which he gave to the Experiment Station project.

To Mr J. R. Gibson, Commissioner of the State Domains, I am indebted for many valuable discussions on cotton problems, and to Mr E. A. Benachi for the examination of the second generation lint samples.

My colleagues in the Society have assisted me loyally with their special knowledge of cognate subjects, and various friends and corre-

spondents in all parts of the world have been most kind in supplying information and seed.

General Conclusions.

1. (*a*) Mendel's Law of Heredity applies to all those characters of the cotton plant which have been critically investigated.

(*b*) Among the allelomorphs concerning which we can as yet advance only provisional hypotheses we find several physiological characters which will enable us to give a definite statistical expression to certain agricultural peculiarities.

2. (*a*) No cases of coupling have yet been discovered which are likely to prevent the synthesis of the desired forms of cotton.

(*b*) The physiological relationships of the plant to the limiting factors of the environment are most important characters from the agricultural point of view, on account of their probable effect on the quality of the lint.

3. (*a*) Most of the characters of the cotton plant fluctuate to some extent, but this fluctuation is far less than has been commonly assumed to be the case.

(*b*) The maximum length of the lint may be forced above the normal by special physiological conditions (either of the environment or of the individual), but it cannot be depressed below that normal.

4. (*a*) Cultivated varieties of cotton in Egypt consist of innumerable different strains of cotton.

(*b*) The deterioration which these varieties undergo after a few years in cultivation is due to this heterogencity through the agency of natural—and unconscious artificial—selection.

5. (*a*) The amount of cross-fertilisation which takes place in the Egyptian cotton fields is not less than five per cent. per annum, nor more than fifteen per cent.

(*b*) The accumulated effect of this annual crossing between the co-existing strains has converted the crop to—or maintained it as—a mass of natural hybrids.

(*c*) This crossing aids in the depreciation of varieties when inferior strains are introduced by seed-mixture.

6. (*a*) Many characters of cotton are intensified on crossing. The causes of this phenomenon are unknown, and the search for them is the most important task in cotton genetics.

(*b*) I cannot resist presenting the speculation that—just as the clovers are hypertrophied and monstrous through the action of nodule bacteria—the improved domestic strains of the higher organisms may be in some respects monstrous through intensification like our cottons.

7. (*a*) On account of climatic and manipulative difficulties, as also the necessity for statistical investigation of the individual, it is impossible to carry out synthetic work in Cotton Genetics unless all facilities for examination of the plants are provided.

(*b*) The Khedivial Agricultural Society has founded the first official Experiment Station devoted to the utilisation of Mendel's Law for economic purposes.

NOTE ON THE ABSORPTION OF ATMOSPHERIC MOISTURE BY CERTAIN NITROGENOUS MANURES.

By GEORGE BROWNLEE, B.Sc.

Albert Agricultural College, Glasnevin, Dublin.

TEN grams each of the manures Sulphate of Ammonia, Nitrate of Soda, Calcium Cyanamide and Nitrate of Lime (Notodden, 1906) were exposed to air in a room the windows of which were kept open during the experiment, and the changes in weight noted for a period of twenty-nine days. At the same time Barometric and Temperature (Wet and Dry Bulb) readings were taken daily.

The percentages of moisture in the manures at the commencement were as follows:—Sulphate of Ammonia 4·52; Nitrate of Soda 2·85; Calcium Cyanamide 0·50; Nitrate of Lime 2·02.

The accompanying chart illustrates the changes in weight and in the atmospheric conditions recorded.

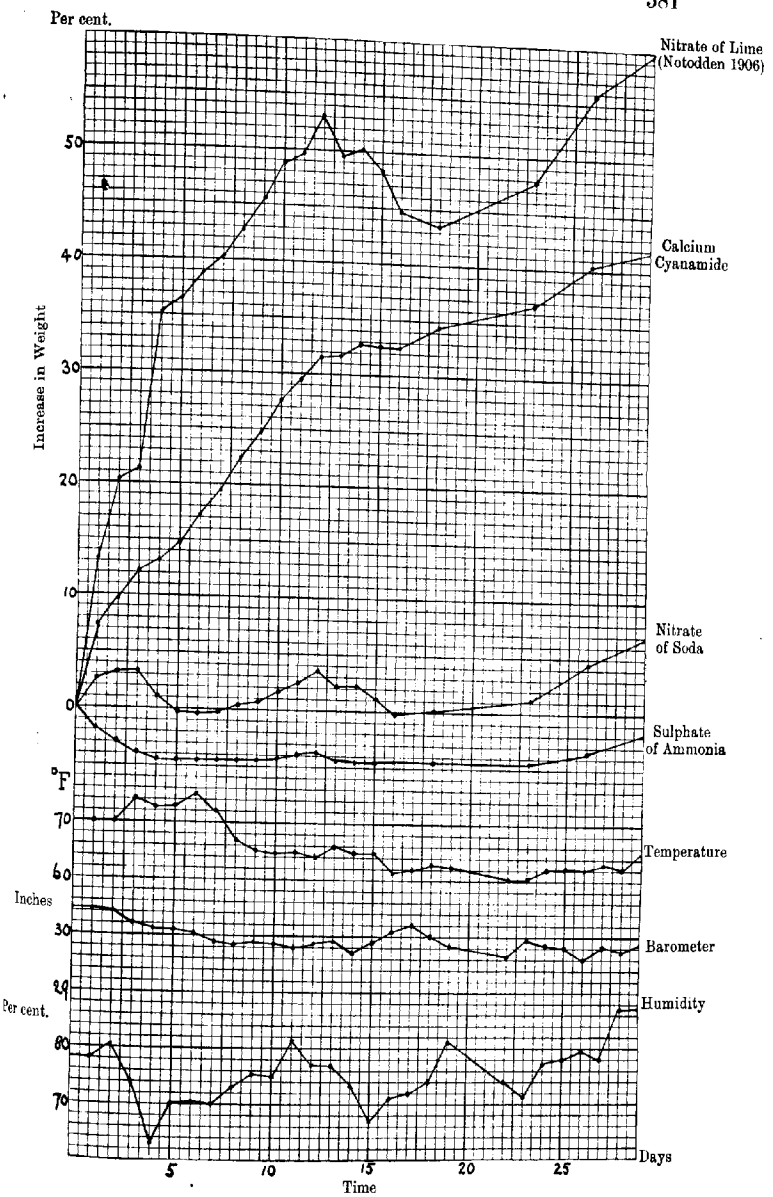
The Sulphate of Ammonia became rather drier during the experiment and was quite powdery at the finish.

The Nitrate of Soda was found at the end to be wettish underneath: apparently its condition varies with the state of the atmosphere. In another experiment on a different sample of the ordinary commercial salt, when the air was very moist for many days in succession, Nitrate of Soda became quite wet. Evidently this manure cannot always be conveniently kept under ordinary conditions.

The Calcium Cyanamide remained unchanged in appearance throughout the experiment. It was, however, partly caked, though quite dry and powdery to the touch. (H. von Feilitzen states[1] that Calcium Cyanamide prepared by the Polzenius process contains a slight excess of Calcium Chloride, which renders the product very hygroscopic, causing it to cake together in hard lumps and to burst the containing sacks.) On opening a bottle containing Calcium Cyanamide there is always a smell of Ammonia showing that this substance would deteriorate if left exposed.

The Nitrate of Lime showed signs of becoming wet after a few hours' exposure and by the third day was quite liquid. It is, therefore, obvious that the Notodden product as made in 1906 must be deemed unsatisfactory from a farmer's point of view.

[1] Abstract in *Jour. Soc. Chem. Indus.*, 26 (1907), No. IX. p. 478.


Per cent.
Nitrate of Lime (Notodden 1906)
50
40
Calcium Cyanamide
Increase in Weight
30
20
10
Nitrate of Soda
0
Sulphate of Ammonia
°F
70
60
Temperature
Inches
30
29
Barometer
Per cent.
Humidity
80
70
5
10
15
20
25
Days
Time

THE COMPOSITION OF GREEN MAIZE AND OF THE SILAGE PRODUCED THEREFROM.

By HAROLD E. ANNETT, B.Sc. (Lond.), S.E.A.C. Dip. *Indian Department of Agriculture* and EDWARD J. RUSSELL, D.Sc. (Lond.) *Rothamsted Experiment Station, late of the South Eastern Agricultural College, Wye.*

Introduction.

The process of making silage is an ancient one[1] and the scientific investigations date back at least to 1873, when Weiske[2], at the Proskau experiment station, showed that there was a loss of carbohydrate, fibre and protein in making silage from sainfoin and other crops. It was, however, by no means general till after the publication in 1875 of Goffart's remarkable success with maize silage at Burtin, in the barren district of Sologne (Loire-et-Cher)[3]. Both Grandeau[4] and Barral[5] analysed Goffart's silage, the former noted the production of volatile and non-volatile acids and labelled them acetic and lactic acids respectively, in which practice he has been followed by most later analysts. Five years later Kellner[6] demonstrated by careful quantitative measurements that the decrease in protein was accompanied by an increase in the amount of "amide" nitrogen. In his experiments about 28 per cent. of the nitrogen was lost, but in a subsequent paper[7] this was traced to volatilisation of ammonia during manipulation of the sample. Kellner considered, and probably correctly, that no nitrogen is lost in the free state from the silo.

Much attention was given to the subject in the wet cycle of years

[1] See *e.g.* Johnston, *Trans. Highland and Agric. Soc.* 1843, new series, 9, 57. A good historical account is given by Jenkins, *Journ. Roy. Agric. Soc.* 1884, 20, 126.

[2] Quoted by Voelcker, *Journ. Roy. Agric. Soc.* 1884, 20.

[3] *Sur la culture et l'ensilage du Maïs-fourrage* (Memoire présenté à la Société centrale d'Agriculture de France, 1875). On p. 8 he says: "Lorsque j'ai acheté le domaine en 1840...huit malheureuses vaches et cent vingt brébis composaient tout le cheptel d'alors et vivaient misérablement...aujourd'hui le même domaine nourrit abondamment soixante-huit bêtes à cornes, six chevaux, et trois cents moutons." He had about 300 acres.

[4] *Ibid.* p. 39. [5] p. 50 and Part 2, p. 24.

[6] *Land. Versuchs-Stat.* 1880, 26, 447. Mangold leaves were used.

[7] Kellner and Sowano, *Land. Versuchs-Stat.* 1889, 37, 16, also Kellner, *Chem. Zeit.* 1890, 14, 905.

ending in 1883: Fry's letters appeared in the *Agricultural Gazette* and *Mark Lane Express* for 1883 and 1884, and were reprinted in book form in 1885[1]; numerous papers, scientific and practical, were published in 1884 and 1885, and in the latter year a commission sat under Lord Walsingham to collect evidence and report thereon[2]. Experiments were also made at Rothamsted[3]. The scientific papers were mainly analytical and did little more than confirm the earlier results. Weiske and Schulze[4] made maize silage in barrels and observed an increase in the amount of ether extract; they supposed this was due to the conversion of sugar into lactic and butyric acids, both of which are soluble in ether. Analyses were published by Kinch[5], Lloyd[6], Richardson[7], Smetham[8], Voelcker[9], and the late Dr A. Voelcker[10], and served to correct a number of misconceptions that had arisen[11]. As silage never became general in England there have been only few papers published here since 1885.

Meanwhile the subject was being taken up in America. In most parts of the States maize is a far more popular crop than roots, and silage provides the stock with their succulent food in winter. A vast amount of work has been done at the different stations, it is only necessary to mention the investigations of Jordan[12] and Armsby[13] on the digestibility, which proved conclusively the loss of nutritive value; Woll[14], Hills[15], Collier[16] and King[17] on the loss of dry matter; King[17] on

[1] *Sweet Silage*, 1885, Agric. Press Co. London.

[2] The Evidence and Report contain interesting accounts of the methods of making silage, its place in the economy of the farm, and its value here and elsewhere.

[3] *Agric. Gazette*, 1885, also *Rothamsted Memoirs*, Vol. 4, No. 12.

[4] *Journ. für Landwirtschaft*, 1883, 32, Heft. 1. Abs. in *Journ. Chem. Soc. Abs.* 1884, p. 1409. Cf. also Palladin, *Ber. der Bot. Gesellschaft*, 1888, 6, 205 and 296.

[5] *Trans. Chem. Soc.* 1884, 45, 122. [6] *Chem. News*, 1884, 49, 210.

[7] *Trans. Chem. Soc.* 1885, 47, 80.

[8] *Journ. Roy. Agric. Soc.* 1884, 20, 360. [9] See *Report of Commission.*

[10] *Journ. Roy. Agric. Soc.* 1884, 20, 482, other workers quoted in this paper are Moser and Holdefleiss.

[11] *E.g.* it was often supposed that fibre became digestible during the process and some even considered that sugar was produced. Lawes, in his evidence before the commissioners pointed out the fallacy of supposing that poor coarse grass, weeds, etc. would change into useful, nutritious food in the silo.

[12] *Maine Reports*, 1893 and 1894 (*Expt. Station Record*, 1895, 6, 746 and 1896, 7, 884).

[13] *Pennsylvania Reports*, 1889 (*Expt. Station Record*, 1892, 3, 457).

[14] *Land. Versuchs-Stat.* 1889, 36, 161.

[15] *Vermont Reports*, 1893 (*Expt. Station Record*, 1895, 6, 919).

[16] *New York State Reports*, 1892 (*Expt. Station Record*, 1895, 6, 65).

[17] *Wisconsin Report*, 1894 (*Expt. Station Record*, 1897, 8, 350 and 687: 1898, 9, 393); also *Wisconsin Report*, 1900.

certain physical questions involved; and Babcock and Russell[1] on the bacteriological aspects of the question.

The composition of green maize.

For some years past it has been the practice at the Wye College to grow green maize as a fodder crop and make a certain quantity into silage. The original idea was to see if silage could to any extent take the place of roots, which, on the light chalky soil of the College farm, are often difficult and sometimes expensive to secure. It may at once be stated that green maize was found to be valuable, but silage was only economical in exceptional seasons[2]. The maize was cut during October as opportunity offered, chopped into pieces, and filled into the silo, a cylindrical wooden structure 12 ft. in diameter and 17 ft. high, standing in an extension of the barn. A large sample of the cut pieces (10 or 15 kilos) was drawn, a small subsample taken for analysis, and the bulk weighed into a sack, sewn up and thrown into the silo. This operation was repeated several times during the filling, so that at the end we had several sacks buried at different depths in the silo, each containing weighed quantities of maize of known composition.

The maize is green when cut and far from being ripe. Its composition depends very much on the season; in warm, dry summers there is about 20 per cent. of dry matter, while in cold, wet seasons only about 13 per cent. is found[3], the difference falls almost entirely on the nitrogen-free extract, so that if the nitrogen-free extract and water are added together the sum is very fairly constant. The nitrogen, fibre, and ash show only slight differences from year to year. Most of the nitrogen (about 80 per cent.) is present as true protein, not much amino acid or amide being present at the time of cutting. The nitrogen-free extract contains a certain amount of sugar, which appears to be mainly dextrose, but no starch. The juice is slightly acid to litmus paper and contains gallic acid, but we could find neither lactic, malic, succinic, nor volatile fatty acids. Distillation with hydrochloric acid caused furfurol to be liberated from the pentosans.

[1] *Wisconsin Report*, 1900.

[2] E. J. Russell, *Journ. Board of Agric.* April, 1907, p. 14. Reference is also made to the feeding value of silage, which is not dealt with in the present paper.

[3] The crop varies in the same way, and is much greater in warm than in cold seasons. The difference in the amount of good stuff per acre is therefore very considerable.

TABLE 1.

Average percentage composition of green maize, end of September and early October[1].

	Good seasons		Bad season	Intermediate seasons		Average of all
	1901[2]	1905[3]	1903[4]	1904[5]	1906[6]	
Dry matter	21·90	18·70	13·35	15·00	15·09	16·81
Ether extract	·85	·52	·16	·66	·22	·48
Total nitrogen × 6·25	1·90	2·04	1·81	1·59	1·58	1·78
Nitrogen-free extract	15·60	9·88	6·70	8·06	8·39	9·33
Fibre	4·25	5·30	3·83	3·74	3·93	4·21
Ash	1·30	·98	·79	·95	·97	1·00
Total nitrogen	·304	·328	·289	·253	·252	·285
Protein nitrogen		·267	·210	·212	·166	·214
Non-protein nitrogen (by difference)		·061	·079	·041	·086	·071
% of total N. present as non-protein		18·8	27·3	16·2	34·1	25·0
Sugar		·98	1·5	·81		1·10
Furfurol obtained		2·48	2·01	1·83	1·92	2·06

The crop is never quite even, it is possible on the same day to pick out large, well-advanced plants with hard, woody pith growing alongside of smaller plants, less mature, with soft pith and much more juicy. The difference in composition lies mainly in the water content, the dry matter being much the same in both.

TABLE 2.

Percentage composition of dry matter of (a) large well-advanced and (b) small less mature plants cut the same day.

	Dry matter	Ether extract	Total nitrogen × 6·25	Total nitrogen	Protein nitrogen	Nitrogen free extract	Fibre	Ash	Furfurol	Sugar
(a) Mature plants ...	18·95	3·24	10·75	1·72	1·52	55·58	25·01	5·42	11·97	5·74
(b) Immature plants.	12·70	4·76	11·00	1·76	1·67	53·29	25·63	5·32	11·74	4·57

[1] For details of the methods employed see the Experimental Part, pp. 389 et seq.
[2] 1 sample. [3] 2 samples. [4] 2 samples. [5] 7 samples. [6] 1 sample.

Composition of Silage.

Some months after filling the silo, the sacks were recovered and their contents weighed and examined. The silage had a brownish-green colour, but otherwise the pieces looked unaltered. It had a pungent smell suggesting butyric acid, and was acid to litmus. Numbers of bacteria were present including *Bac. subtilis* and others, but there was no mould of any sort. The composition is curiously constant, showing far less fluctuation from year to year than does the original maize.

TABLE 3.

Average percentage composition of maize silage.

	1901	1903	1904	1905[1]	1906	Average of all
Dry matter	12·10	12·20	13·79	13·56	13·32	12·99
Ether extract[1]	·16	·10	·83	·56	·29	·39
Total nitrogen[1] × 6·25	1·33	1·35	1·65	1·69	1·30	1·45
Nitrogen-free extract	5·08	5·67	5·15	5·32	5·61	5·38
Fibre	4·66	4·13	5·06	5·06	5·20	4·82
Ash	·98	·95	1·10	·93	·92	·98
Total nitrogen	·213	·216	·265	·269	·208	·234
Protein nitrogen		·121	·156	·181	·089	·137
Non-protein nitrogen (by difference)		·095	·109	·088	·119	·103
% of total N. present as non-protein		43·8	41·1	32·8	57·2	43·72
Nitrogen as NH_3		·003	·006	·014	·006	·007
Nitrogen as amide			·007	·010	·001	·006
Sugar	nil	nil	nil	nil	nil	nil
Furfurol obtained		2·00	1·87	2·26		2·04
Volatile acid (as H_2SO_4)		·17	·07	·03		·09
Non-volatile acid (as H_2SO_4)		·30	·68			·49

When silage is pressed it readily yields quantities of a brownish juice of very complex composition. The amount and nature of the nitrogen compounds in 100 c.c. of a typical sample of this juice were as follows:

Total nitrogen	·161	
Nitrogen as NH_3	·030 = 18·6 %	of total
Nitrogen as amide	·014 = 8·7 %	,,
Nitrogen as amino acid	·089 = 55·3 %	,,
Nitrogen not accounted for	·028 = 17·4 %	,,

[1] The figures for total and non-protein nitrogen and ammonia are all somewhat too low because we have not been able to avoid loss of ammonia during sampling. We reduced loss as far as possible by starting the several nitrogen determinations in the wet silage immediately the sample was drawn. The ether extract figures are only approximate. See experimental part for details.

A list of the more important compounds present in silage appears in the accompanying paper. It comprises a number of acids containing no nitrogen, which probably have no actual feeding value; among these are formic, acetic, butyric, caproic and hexoic acids, two hydroxyacids, lactic and malic, and also succinic acid. There are also simple nitrogenous compounds, amino acids, basic diamino acids and amides, the feeding value of which is not yet settled, but is certainly less, and probably much less, than that of protein. Finally there are amines which are actually injurious, though whether they normally occur to a sufficient extent to do any harm may be doubted; cases have come to our notice, however, where animals have not thriven on silage and the trouble may very probably be ascribed to these bodies.

It is common to speak of the large amount of "amides" present in silage, but Table 3 shows how very small the quantity really is. Amides occur to a smaller extent than ammonia, and to a still smaller extent than amino-acids. They form only about 5 per cent. of the non-protein nitrogen, and in view of this fact it is highly desirable that the practice of regarding all of the non-protein nitrogen compounds as amides should be discontinued.

The losses going on during ensilage.

On comparing the composition of silage with that of the maize from which it was formed it is possible to discover what has been the loss during the process. An exact quantitative comparison is rendered impossible by the difficulties of sampling and of preventing loss of ammonia, but it is quite clear that a considerable amount of dry matter has disappeared during the process. The loss is not uniform throughout the silo, and no two bags give exactly the same results, but the average of our experiments during 1905 and 1906, set out in Table 4, shows its general distribution.

It will be noticed that the fibre is practically unaltered in amount, and we have confirmed this observation by microscopically examining sections of maize and of the silage afterwards produced. We found that the epidermal cells had undergone no change in the silo beyond a certain amount of shrinkage; even the stomata were unaltered. Similarly the vascular bundles were intact.

The nitrogen-free extract suffers most. Direct tests showed that the sugar disappears almost entirely. The fall in the furfurol indicates a decrease in the amount of cellulose, *i.e.* the less resistant cellulose, which alone falls into this group; this was confirmed by microscopic

examination which showed that many of the cells of the mesophyll were completely disintegrated.

The protein suffers considerably, though not in reality as much as the nitrogen-free extract. Hydrolytic decomposition complicated by bacterial action takes place, and although there is not much, if any, loss of free nitrogen the new nitrogenous compounds are less valuable as food than the protein.

TABLE 4.

Mean losses in the silo 1904 *and* 1905.

	Dry matter	Ether extract	Nitrogen free extract	Fibre	Total nitrogen	Protein nitrogen	Non-protein nitrogen	Ash	Furfurol
Put in	100	100	100	100	100	100	100	100	100
Brought out..	64	84	45	92	74	45	183	86	68
Loss.....	36	16	55	8	26	55		14	32
Gain.....							83		

The figures show an absolute loss of nitrogen, but we are satisfied that a certain amount of ammonia is given off during sampling, and we have no evidence to show how or to what extent losses of nitrogen occur in the silo. In laboratory experiments on silage made in bottles we only observed losses of nitrogen when air was admitted, and this did not happen in the silo.

The figures given for the loss in ether extract are quite accidental, since the value obtained in any particular bag depends on its position in the silo; the bags in the *top* half showed a considerable loss—32 per cent. on the average—those in the *lower* half showed an average gain of 6 per cent. The ether extract of silage contains a number of soluble acids which obviously wash downwards. Further, since these acids are derived from the nitrogen-free extract or protein of the maize it follows that no comparison is in any case possible.

A similar downwash occurs with the soluble ash constituents, the upper bags losing on an average 17 per cent., and the lower bags gaining 2 per cent., hence the average figure given in Table 4 is of no value. The insoluble ash constituents are of course not liable to this movement; but their amount is so small and variable—depending

partly on the presence of stray soil—that the experimental error becomes too large to give the calculations any value.

The general nature of the losses outlined above is probably the same in all silos, though the actual amount varies: our losses are higher than those observed by American investigators, but there is considerable difference between American and English maize at the time of cutting; ours is much less mature, and contains a lower proportion of nitrogen-free extract.

When a farmer makes silage on a large scale he often wants to know what loss takes place in his silo. The most convincing way of demonstrating this is, of course, to bury weighed bags of material as we have done, but a sufficiently accurate result can be obtained by assuming that the fibre undergoes no change, or only a 5 per cent. loss, and calculating on this basis the amount of each constituent that should be present. This method is much better than another which is sometimes used—viz. to assume that the ash is unchanged in amount and to use it as the basis of calculation; in the first instance, as we have just seen, the soluble ash is liable to wash downwards, and in the second place the percentage of ash is so small that a trifling error in sampling or determination very considerably affects the result. Of course there is in any case the difficulty of getting the average composition of the green material and the silage, which is only got over by taking a number of samples. In our experiments more than one-third of the total dry matter was lost, including more than half the nitrogen-free extract and the protein; in the latter case some of the decomposition products, the amino-acids etc. remained in the silo, so that the actual loss of nitrogen was only 26 per cent. The fibre appeared to undergo no change.

Experimental details.

Analytical methods. The determinations of fat, total nitrogen, fibre, etc., were all made in the ordinary manner. Non-protein nitrogen was estimated by Stutzer's method, the substance is boiled with a mixture of copper hydrate and glycerine which dissolves the non-protein but leaves the protein in the insoluble residue. The method is no doubt open to objection because some proteins may dissolve and some insoluble non-protein bodies, *e.g.* the purin bases, may remain insoluble. But when used for purposes of comparison and not with an idea of getting absolute results we consider that it gives quite valuable information.

To determine the quantities of nitrogen present as ammonia, amide, and amino-acid respectively we adopted the very elegant methods used by Drs Horace Brown and Millar, described in the *Transactions of the Guinness Research Laboratory*, Vol. I. Part 1, 1903. We have found them work very satisfactorily with our subtances.

The furfurol obtained on distillation with hydrochloric acid was converted into its hydrazone and weighed. Krug's method of working was adopted, but we prefer not to attempt expressing our results in terms of any particular pentosan. It is well known that several groups of bodies give furfurol on distillation, including pentosans, celluloses (the so-called "oxy-celluloses"), glycuronic acid, etc., but that certain members of the groups yield instead non-volatile hydroxy-furfurols, and hence cannot be estimated in this way. In view of the fact that the furfurol yielding bodies of green maize have not been carefully examined we prefer to give the experimental figure only, and in the Tables have given the actual weight of furfurol obtained from 100 parts of substance. No doubt the less resistant or "oxy-celluloses" are responsible for much of the furfurol.

Method of procedure. The maize was cut up, roughly dried at about 80° C, ground in a coffee mill and reduced to a fine powder in the Maercker mill. Drying could now be completed, and samples taken for analysis.

A different scheme had to be adopted for silage. As the bags were recovered from the silo they were weighed, brought to the laboratory, and samples taken as speedily as possible for the various nitrogen determinations. But no matter how quickly we worked we could not altogether avoid loss; our figures for total nitrogen and free ammonia are therefore low. Still, the loss is much less than if the samples had been dried before the nitrogen was determined, for the nitrogen found in the dried material is always less than one expects by calculating from the amount in the original wet silage. It will be observed that in three cases the difference is approximately equal to the amount of ammonia present in wet silage, but in the other cases it is much greater.

Samples 1 to 4 were drawn from the 1905 silage, sample 5 was taken in 1906, and sample 6 comes from a silo on another farm.

Another sample of silage was quickly drawn for the determination of the volatile acid. It was distilled in steam, and the distillate titrated with standard alkali. The results are only approximate, the higher acids come over so slowly that the distillate never really becomes

neutral. The non-volatile acid was estimated by titrating the residue in the flask, but we soon gave up this determination because of the difficulty of getting a sharp end reaction.

TABLE 5.

Percentage of nitrogen in the dry matter of silage.

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Calculated	1·90	2·10	2·41	2·15	1·58	2·31
Found	1·42	1·61	1·19	1·24	1·45	1·70
Loss during drying	·48	·49	1·22	·91	·13	·61
Ammonia found in wet silage...	·41	·36	·33	·94	·01	·07

A third sample was dried, ground, and used for the estimation of ether extract, fibre, ash, and nitrogen-free extract. The ether extract results are only approximate, samples continue losing weight for days in the extraction apparatus. Some of the acids are only slightly soluble in ether, the colouring matter also dissolves with difficulty.

The bulk of the sample was then used for qualitative examination.

THE CHEMICAL CHANGES TAKING PLACE DURING THE ENSILAGE OF MAIZE.

By EDWARD J. RUSSELL, D.Sc. (Lond.).

Rothamsted Experiment Station.

When the green parts of living plants are cut up and packed in a loosely covered vessel allowing entrance of air, mould soon makes its appearance and decomposition begins: the mass becomes alkaline and is ultimately converted into black humic bodies quite unfit for cattle food. But if air is excluded the change is fundamentally different; no mould developes, the temperature rises, the mass takes on a greenish-brown colour and characteristic odour, it becomes acid and for a long period is suitable for cattle food. The former is a putrefactive change, the latter gives rise to silage.

The general chemical changes known to take place during ensilage are the conversion of sugar and similar bodies into carbon dioxide and water, the production of volatile acetic and butyric acids and of non-volatile lactic acid and the conversion of protein into non-protein[1] material.

Several hypotheses have been put forward to account for the silage changes and to explain why the product keeps so long. According to one—perhaps the commonest—fermentations set up by micro-organisms evolve so much heat that everything is killed and the mass becomes sterilised. Another view is that certain thermophilous organisms bring about the observed changes[2]. Wollny[3] considered that the lactic and

[1] Sometimes called "amides," but it is highly desirable that this term should be dropped. The word "amide" has a definite chemical significance, and many of the nitrogenous non-protein bodies in plants are not "amides" but amino-acids, etc.

[2] *E.g.* Griffiths, *Chem. News*, 1894, 70, 273: see also Lafar, *Tech. Mycology*, p. 262.

[3] *Die Zersetzung der Organischen Stoffe*, 1897.

acetic acids were formed by bacteria but that the rise of temperature was a respiration effect. On the other hand Pasteur's work on anaerobic respiration led Fry[1] to conclude that the changes are not due to bacteria at all but to the cell, and are the result of the altered conditions in which the cell now finds itself. In absence of air oxidation of sugar does not go as far as carbon dioxide and water, but stops at alcohol and acetic acid. This view has been developed by Babcock and H. L. Russell[2] in an important paper published in 1902; they found that silage could be made perfectly well in the presence of ether or chloroform and they therefore conclude that bacterial activity cannot be an essential factor in the process. No acid was produced in this case, however; but acidity appeared when the cells were not killed and the longer the cells lived the more acid was formed.

The first of these hypotheses may be dismissed at once, since silage invariably contains bacteria and is never sterile. Bacteria must therefore obviously play some part, even if only a secondary part, in the process. It is equally clear that the living cell is an active agent. Silage is always made from living plants and the cells live for some time after they are put into the silo; the breaking down processes can still go on though they may be modified by the absence of air, but the building up processes depending on light and air are stopped. Three sets of agents, the living protoplasm, enzymes and bacteria, appear to be involved, and no hypothesis is satisfactory which fails to take account of all three.

The green maize and the silage dealt with in the present paper were produced at the Wye Agricultural College, where a large part of the experimental work was done. The method of making the silage is described in the preceding paper; the details of the separation and the analytical results are in the experimental part of the present one.

The substances produced during ensilage.

On comparing the weights of the various groups of constituents of green maize put into the silo with those of the silage brought out it is found (see preceding paper) that (1) there has been practically no change in the fibre, (2) all of the sugar and some of the less resistant celluloses disappear, (3) carbonic acid is evolved and a number of acids appear, which were not before present, (4) the "protein" nitrogen

[1] *Sweet Silage*, 1885, Agric. Press Co. London.
[2] *Centr. für Bakt.* 1902, 9, 81.

compounds, *i.e.* those forming insoluble compounds with copper hydrate, are reduced to about one-half, (5) the non-protein nitrogen compounds, *i.e.* those forming soluble copper compounds, practically double in amount.

The changes undergone by the nitrogenous compounds are well seen in the juices pressed out from maize and from silage respectively; the latter contains more substances reacting with nitrous acid and with phosphotungstic acid than the former.

	Weights in 100 c.c. of juice		Relative weights, total N. = 100	
	Fresh maize juice, grams	Silage juice, grams	Fresh maize juice	Silage juice
Total Nitrogen.....	·0677	·161	100	100
N. liberated by HNO_2[1]	·0183	·089	27	55
N. precipitated by phospho-tungstic acid.....	·0108	·060	16	37

The relative amounts of the different groups of nitrogen compounds in silage vary somewhat, but the juice referred to in the preceding table contained in 100 c.c.:

	Grams	Percentage, total = 100
Nitrogen as ammonia	·030	18·6
Nitrogen as amide	·014	8·7
Nitrogen as amino-acid	·075	46·5
Nitrogen not accounted for...	·042	26·2
Total...	·161	100

These results indicate the general nature of the changes; we can, however, get more definite information by ascertaining what compounds are actually present. The following have been isolated by the author from silage:

Alcohols	*Fatty acids*	*Hydroxy-acids*	*Dibasic acids*
Ethyl alcohol	Formic acid	Lactic acid	Carbonic acid
	Acetic acid	Malic acid	Succinic acid
	n-Butyric acid		
	Isopropylacetic acid		
	Hexoic acid (*either* *n*-caproic *or* isobutylacetic acid)		

Nitrogen compounds

Mono-amino acids	*Diamino acids*	*Basic compounds*
A mixture which could not be separated. Reactions indicate	Histidine	Ammonia
	Lysin	Pentamethylene diamine
	Ornithin	Betain (trace only)
glycocol		Adenin
alanine and others		Another purin base not identified

[1] In the apparatus designed by Drs Brown and Millar (*Trans. Guinness Research Lab.* 1903, 1, 30).

In addition there was some gummy matter, some lecithin, a fragrant oil, a little humus, besides the usual celluloses, protein, oil, etc.

The following were sought for, but could not be found: aldehyde, higher alcohols, glycerin, arginin.

Many of these are altogether absent from maize, *e.g.* the volatile acids, lactic acid, etc.; others are found, *e.g.* the amino acids, but to a much less extent.

This list does not include all the constituents, for indications of other bodies were obtained; but it will be found sufficient to give a fairly accurate idea of what goes on in the silo.

The agents producing the changes.

It has already been stated that there are three possible agents likely to be involved in bringing about changes in the silo, viz., living protoplasm carrying on its vital functions particularly respiration, enzymes which, though originating from protoplasm, can nevertheless act independently of it, and micro-organisms. The relative parts played by these three cannot be ascertained by direct experiment because of the impossibility of sterilising pieces of living maize, hence the effect of the cell alone, apart from organisms, cannot be investigated. The method adopted in the first series of experiments was to pack into bottles (which make very good miniature silos) pieces of (A) living maize, to get the total effect of all three agents, (B) maize killed and sterilised with toluene, which still allows enzymes to act, (C) maize killed by heating to 98° C., when enzymes and living organisms are destroyed but not spores; certain organisms may therefore develope, (D) living maize inoculated with silage juice containing large numbers of the organisms actually occurring in the silo. In all these cases the bottles were closed to prevent entrance of air but provision was made for the escape of gases. Further, in order to observe the effect of admitting air some of the bottles containing living maize were simply plugged with cotton-wool (E). The bottles were then kept for five months at 20° C. in an incubator. At the end of the period the bottles were opened and the contents examined. The results may be summarised as follows:

Air excluded				Air admitted
A Protoplasm, enzymes and casual organisms all acting. (Living maize)	B Enzymes only acting. (Maize and toluene)	C Protoplasm and enzymes killed: spore-forming organisms present. (Maize heated to 98° C.)	D Protoplasm, enzymes and silage organisms present. (Maize and silage juice)	E (Living maize)
Silage formed	No obvious change	No obvious change	Silage formed	Putrefaction
No mould	No mould	Little mould	No mould	Much mould
Some dry matter lost (25 %)	No loss	Little loss (12 %)	Some loss (25 %)	Much loss (60 %)
Mass becomes acid	No change	No change	Mass becomes acid	Mass becomes alkaline
Volatile acids (acetic and butyric) formed	None formed	None formed	Acetic and butyric acids produced	None formed
Protein changes to non-protein	Protein changes to non-protein	No change	Protein changes to non-protein	Non-protein changes to protein
No loss of nitrogen	No loss of nitrogen	No loss of nitrogen	No loss of nitrogen	Nitrogen lost

It will be observed that the characteristic silage changes, the production of the silage odour and colour, of volatile acids and the conversion of protein to non-protein, occur only in A and D where living cells are kept out of contact with air. If cells and organisms are killed, but enzymes are allowed to act, only one of the changes takes place—the conversion of protein to non-protein. If spore-forming organisms alone survive, as in C, none of the silage changes occur.

The volatile acids. Since these are formed only in A and D and not at all in B or C their formation must be attributed either to the living protoplasm of the maize cell or to casual organisms; their invariable and rapid production in A (this experiment was repeated a number of times) appears strongly to indicate that they are formed by the living cell. Further, their non-occurrence in E shows that they arise only when the living cell is deprived of oxygen. They may therefore be regarded as products of anaerobic respiration. But although the living cell is probably the primary agent, the organisms also play a part in either producing or modifying them; the acids in D, where organisms were introduced, were somewhat different in character from those in A.

Complete separations were not attempted, but the silver salts of the mixtures contained the following percentages of silver:

	Mixture in A (living maize only)	Mixture in D (living maize + silage organisms)
Bottle 1.....	56·56	60·21
Bottle 2.....	57·52	

Silver acetate contains 64·65 per cent. and silver butyrate 55·40 per cent.

It appears that more of the lower homologues are present in D than in A.

The mixture found in the silo consists mainly of butyric and acetic acids, but there occur also formic acid and some of the higher acids. Formic acid and the higher acids are known to be the products of the bacterial decomposition of cellulose, and as cellulose disappears during ensilage one can safely attribute part of the volatile acid to organisms. These decompositions are, however, quite secondary.

The conversion of protein to non-protein. Unlike the formation of volatile acids the decomposition of protein does not depend directly on the living cell, for we find it going on in B, where the cells had been killed by toluene. It does not, however, occur in C, where the enzymes had been decomposed, and this change is therefore to be attributed to enzymes which can act not only during the life, but also after the death of the cell. The amount of change observed was:

	A (maize only)	B (maize and toluene)
Protein N. put in	100	100
Protein N. brought out	75	89
Protein N. converted into non-protein...	25	11

The quantity of protein decomposed is less when the cells are suddenly killed by toluene than when they continue living for some time, but this is quite consistent with the general facts of enzyme action. The killed cells contain only the amount of enzyme present at the moment of death, action must therefore stop as soon as this has acted on the protein in its immediate vicinity; moreover the toluene may retard its action. In A, on the other hand, fresh quantities of enzyme can always be made so long as the cell lives, and the presence of a little acid is known to be favourable to protein hydrolysis.

Further evidence of the presence of enzymes capable of decomposing protein was obtained by studying the changes in sterilised maize juice. Some green maize was cut in October at the time the silo was being filled, it was pressed, and the juice, to which 2 per cent. of toluene was added, was kept at 25° C.; after some time it was found to have undergone a considerable amount of hydrolysis, for there was a great increase in the compounds decomposed by nitrous acid and in those precipitated by phosphotungstic acid. Action has been more vigorous than in the last experiment, probably because diffusion has gone on better.

	In fresh maize juice	After being kept at 25°
Total N.	100	100
N. liberated by nitrous acid	27	55
N. in phosphotungstic acid precipitate	16	33

As 2 per cent. of toluene was found to keep the juice sterile (for no growth was obtained on inoculating a little into bouillon) this hydrolysis can only be attributed to enzymes. It is therefore evident that the maize put into the silo contained enzymes capable of hydrolysing the protein of the cell even after the cell is dead.

The change that has gone on in the silo clearly indicates that such a hydrolysis has actually taken place and is to be regarded as the primary cause of the decomposition of protein during ensilage. In the first place the average general grouping of the nitrogen compounds in silage (see p. 394) is much the same as that in the hydrolysed maize juice just referred to; nitrous acid liberated 55[1] per cent. of the total nitrogen in each case, and phosphotungstic acid precipitated 37 and 33 per cent. respectively. In the second place typical products of proteolysis occur in the silo. When protein is hydrolysed by tryptic enzymes the products include mono- and di-amino acids (lysin, histidin, etc.). When nucleo-proteins are hydrolysed the purin bases are obtained in addition. All these compounds are found in silage.

It would not, however, be correct to ascribe the whole change to enzymes. The organisms present are not without action on the nitrogen compounds, and certain bodies, *e.g.* amines characteristic of bacterial action, are found in the silo. But the bacterial changes appear to be secondary and not an essential part of the process.

[1] 55 per cent. was the mean amount obtained from silage juice; the actual amount, however, varied considerably in different samples from 38 per cent. to about 65 per cent.

The influence of free oxygen on the process. The effect of allowing free oxygen to have access to the maize in E (p. 396) was to alter the product completely. There was a great development of Penicillium, which did not appear when air was excluded. The mass became black and had a musty smell, it was alkaline and appeared to be free from acetates and butyrates; there had been an increase in the amount of protein, indicating that some had been formed from the simpler non-protein material, presumably by the mould. The general changes were:

	Dry matter	Protein nitrogen	Non-protein nitrogen	Total nitrogen
Put in	17·95	·197	·104	·301
Brought out	7·00	·238	·041	·279
Loss.....	10·95	−·041	·063	·022
Percentage loss ...	61·0		61·5	7·3
„ gain...		20·8		

It will be remembered that in A and B where air was excluded, the protein had decreased 25 and 11 per cent. respectively, whilst here it has increased 21 per cent.

The change in the non-protein substances was also shown directly by determining the amount of nitrogen liberated by nitrous acid. In the case of good silage juice more than 40 per cent. of the total nitrogen is set free by this reagent; while only seven per cent. is liberated from juice expressed from the black, alkaline mouldy layers found at the top of the silo, stretching down as far as the air can enter. Again, some fresh silage juice, which gave up 38 per cent. of its nitrogen on treatment with nitrous acid was allowed to become mouldy; after three months only 18 per cent. was set free.

The disappearance of non-protein, and the formation of protein, indicates that the moulds and other organisms have utilised the simpler substances as food, and built them up into complex cell constituents. A similar change is known to take place when farmyard manure is stored.

The chemical effects following on the admission of air can be ascribed to the course of respiration in the cell, which now remains normal, so that butyric and acetic acids are no longer formed in quantity, and to the development of mould, which appears in the black decomposed layers at the top of the silo as far as oxygen can get in but not lower

down. Indeed the absence of mould is characteristic of good silage, though it is very difficult to explain. Penicillium is reckoned among the hardiest forms of life, it seems able to grow almost anywhere and to tolerate bodies that would be fatal to most other organisms, yet it cannot grow in the silo, and at a certain distance down it stops absolutely short. On the dividing line where the black mouldy layer ends and the good silage begins the author has often found pieces of maize one inch long, one end of which was strongly acid and free from mould, while the other was alkaline and had a growth of Penicillium. It would appear that there is some actual inhibiting agent produced in the silo when oxygen is absent and not formed when oxygen is present, though no doubt the absence of free oxygen in the silo is also a cause why mould does not develope.

The course of the process in the silo.

Putting together our results it is possible to sketch out fairly completely what happens in the silo. When the cells are put in they are alive and their vital functions continue. Respiration goes on and sugar, etc., is used up, but in absence of air oxidation is not complete, and intermediate bodies, alcohol, acetic acid, butyric and other acids are formed in addition to carbon dioxide and water. The tryptic enzymes of the cell act on the protein forming the usual hydrolytic products, amino acids, diamino acids, etc.: from the nucleo-proteins purin bases are produced in addition. The heat developed during these processes cannot be dissipated, as it usually is in the living plant by the evaporation of water, because water vapour cannot escape from the silo; instead it raises the temperature of the mass. Respiration is accelerated by the increased temperature, but, as no more material is being elaborated and only decomposition is taking place the process soon comes to an end; the cell then dies for want of more substance to break down, it loses its turgidity, and becomes flaccid, causing the mass to settle down. The temperature also steadily falls[1]. The decomposition of the protein, which had also been accelerated by the rise in temperature, can continue even after the cell is dead, because the enzymes when once formed are not dependent on the life of the cell, in other words, it is an autolytic decomposition. Some of the products formed in the above changes inhibit the development of mould, and the general conditions obtaining

[1] The maximum of 38° to 37° C. observed in the Wye silo was always reached in about 5 days; there was then a slow but regular fall.

in the silo are unfavourable to putrefaction. The mass therefore remains good for food for a long time.

It is, however, not sterile. Certain bacteria are always present and attack the softer celluloses (but not to any extent the resistant "fibre") producing the humus, some of the fatty acids, and probably the succinic acid present in silage. They also carry several stages further the decomposition of certain of the nitrogen compounds, and produce those bodies which cannot be supposed to arise from tryptic hydrolysis of protein. Thus pentamethylene diamine is a well-known decomposition product of lysin; ornithin is known to arise from arginin, and the absence of the latter body may be due to its decomposition into ornithin and urea, which would further change into ammonium carbonate.

In the following scheme are set out the changes as the author supposes them to take place. The lactic and malic acids might have arisen from the amino acids or from the carbohydrate material, the present experiments do not enable one to decide this point.

On the above hypothesis the changes essential to the production of silage—the disappearance of sugar, the formation of volatile fatty acids and the hydrolysis of protein—are all considered to be the result of the activity of the living protoplasm or of the enzymes of the maize cell. The other changes due to the bacteria invariably present are considered to be of purely secondary importance and merely have the effect of complicating the product without greatly altering its nature or even masking the primary changes.

EXPERIMENTAL PART.

ISOLATION AND IDENTIFICATION OF THE COMPOUNDS PRESENT IN SILAGE.

1. *Non-nitrogenous compounds.*

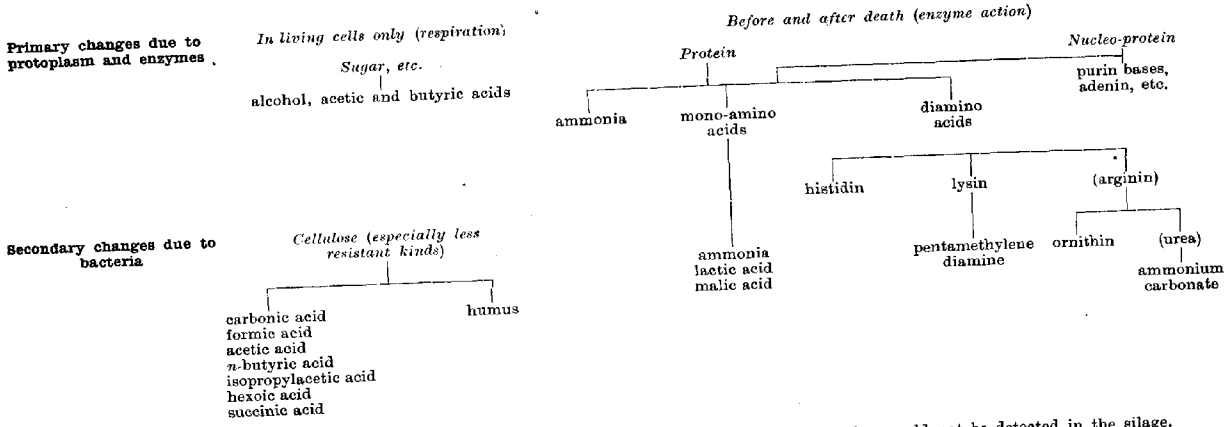
Ten kilos of silage were distilled in steam and ten litres of distillate collected. Towards the end of the process ·5 per cent. of sulphuric acid was added to set free any combined acids.

The distillate (A) contained alcohol, volatile acids, etc.

The residue in the flask (B) contained non-volatile acids.

Examination of distillate A. Excess of silver oxide was added to

SCHEME SHOWING CHANGES DURING ENSILAGE.



The substances in brackets are supposed to have been formed as intermediate products, though they could not be detected in the silage.

combine with the acids and the whole was distilled. The distillate (C) contained non-acid bodies, the residue (D) was composed of silver salts of the volatile acids.

The non-acid bodies in C. This had the characteristic maize smell. A portion tested with Schiff's reagent gave no colour. Aldehyde is therefore absent.

Another portion was treated with potassium permanganate and sulphuric acid. Aldehyde was formed, it was distilled off and gave an intense Schiff reaction. A **primary alcohol** is therefore present.

On treatment with iodine and sodium hydrate a precipitate of iodoform was obtained. The crystals were seen under the microscope to form the star-shaped clusters characteristic of **ethyl alcohol**[1].

Six litres of the distillate were evaporated on the water bath, there was a residue, but it contained neither glycol[2], glycerine[3], nor fusel oil[4]. It gave no aldehyde on oxidation, but yielded an acid which, however, was too small in amount for investigation; it was not oxalic acid.

The rest of the distillate was extracted with ether previously washed and distilled over sodium to remove alcohol. After distilling off the ether a small pleasant smelling residue was left which was apparently an ester. There was no evidence of fusel oil.

The **absence of glycerine** and **higher alcohols**, except in traces, is therefore probable.

The volatile acids in D. The silver salts were extracted with hot water and boiled for a few minutes. Reduction took place, indicating the presence of **formic acid.** This was confirmed by treating a new lot of distillate with mercuric chloride and digesting at 90° C. for some time, when mercurous chloride was precipitated.

The solution of silver salts was filtered, concentrated, and the crystals taken out in four fractions. Each fraction was similarly broken up into two others which were purified by recrystallisation, the separate fractions being united when they were evidently identical.

The first crop of crystals was small, and was found to be a silver **hexoate,**

Ag found = 48·74 per cent., $C_6H_{11}O_2Ag$ requires 48·43 per cent.

[1] A large number of bodies react with iodine and sodium hydrate to form iodoform, but under the high power of the microscope this is seen to form hexagonal plates excepting when ethyl alcohol is present, in which case the crystals are star-shaped.

[2] Glycol gives oxalic acid on oxidation.

[3] Dunstan's test was used, *Pharm. Journal* [3], vol. 14, p. 41.

[4] The higher primary alcohols give aldehyde on oxidation. Fusel oil is insoluble in ether and this substance readily dissolved.

The intermediate crops yielded a little **valerate**.

Ag found = 51·9 per cent., $C_5H_9O_2$ Ag requires 51·68 per cent., and a large amount of a **butyrate**,

Ag found = 55·83 per cent., $C_4H_7O_2$ Ag requires 55·40 per cent.

The last crops yielded silver **acetate**,

Ag found = 64·40 per cent., $C_2H_3O_2$ Ag requires 64·65 per cent., while the mother liquors contained a small amount of what appeared to be silver lactate.

It is not possible, however, to discriminate between the isomers by means of the silver salts. In order to do this a fresh lot of the acids was prepared from silage and converted into sodium salts; these were dried, well ground and suspended in alcohol, a current of hydrochloric acid gas was then passed through the mixture. The esters thus obtained were fractionated, and the separate fractions on redistillation yielded small amounts of liquids with fairly constant boiling points. The liquids were identified by molecular weight determinations in a Victor Meyer's apparatus.

The hexoic ester was found to be either *n*-**caproic** or **isobutyl acetic** ester.

Mol. wt. found = 139, theor. 144. B. pt. of fraction = 160°—170°. B. pt. of isobutyl acetic ester = 161°, of *n*-caproic ester = 167°.

The small amount of hexoic ester was insufficient for further examination and it is not possible to say which of the two isomers is present.

The valeric ester was found to be either methyl ethyl acetic ester or isopropyl acetic ester.

Mol. wt. found = 126, theor. 130. B. pt. of fraction = 132°—135°. B. pt. of methyl ethyl acetic ester = 133·5°, of isopropyl acetic ester = 135°.

The ester was dissolved in chloroform and examined in a polariscope. It was found to be optically inactive and is therefore **isopropyl acetic ester.**

The butyric ester was found to be *n*-**butyric** ester.

B. pt. found = 119°—121°. B. pt. of *n*-butyric ester = 120·9°.

Small amounts of a high boiling substance were found, and were probably ethyl lactate.

Mol. wt. found = 119. Mol. wt. of ethyl lactate = 118. B. pt. found = 150°—160°. B. pt. of ethyl lactate = 154·5°.

The non-volatile acids in B. The liquid was acidified with sulphuric acid, decanted from the silage, and extracted repeatedly with ether.

The ether was then distilled off, water was added and calcium carbonate in excess, and the calcium salts obtained were evaporated to dryness on the water bath. They were then extracted with 90 per cent. alcohol, and the residue was treated with water. Part remained fairly insoluble.

(*a*) *The salt soluble in alcohol.* This had a brown colour and could not be crystallised, it was accordingly dissolved in alcohol and precipitated by ether. Repetition of the process two or three times gave a white salt crystallising in the tufts of needles characteristic of calcium **lactate**. This was dried at 100° and analysed,

CaO found = 25·50, calculated for $Ca(C_3H_5O_3)_2$ 25·69.

(*b*) *The salt readily soluble in water.* This also was brown; it was dissolved in a small amount of water and precipitated by alcohol, the process being repeated several times. The pure salt was found to be calcium **malate**. It was dried at 100° and analysed,

CaO found = 29·30, calculated for $CaC_4H_4O_5 . H_2O$ 29·47.

As malic acid had not before been found in silage we confirmed its presence by preparing and analysing the silver salt, this being made from the calcium salt,

Ag found = 61·63, calculated for $Ag_2C_4H_4O_5$ 62·07.

Further confirmation was obtained by preparing the lead salt, and by isolating the acid and converting it into malic acid.

(*c*) *The salt less soluble in water.* This differed from the previous salts in giving a buff coloured precipitate with ferric chloride. Analysis of the salt dried at 100° showed it to be calcium **succinate**.

CaO found = 32·32, calculated for $CaC_4H_4O_4 . H_2O$ 32·18.

(*d*) *The residue insoluble in water.* This contained neither oxalic nor tartaric acids, and appeared to be only calcium carbonate with a little sulphate.

2. *The nitrogenous compounds.*

As it was only proposed to seek for soluble nitrogen compounds the author used the juice squeezed out from fresh silage, and adopting a slight modification of Schulze's method as described in *Landw. Versuchs-Stat.* 1904, Vol. 59, p. 344.

1. The juice was first treated with basic lead acetate[1] to precipitate

[1] Prepared by boiling a 30 % solution of normal acetate with excess of finely ground litharge, allowing to cool, and filtering.

the finely divided matter floating in the liquid and also the gummy bodies vaguely known as extractives. The precipitate (A) was filtered.

2. The filtrate from A was treated with hydrogen sulphide to remove lead, then concentrated and treated with a 10 per cent. solution of phosphotungstic acid in five per cent. sulphuric acid. The precipitate (B) may contain the basic diamino acids, arginin, histidin, lysin, and ammonia, amines, betain and cholin.

3. The filtrate was treated with mercuric nitrate and caustic soda was added till the solution was faintly alkaline. The precipitate (C) may contain mono-amino acids, ureids (*e.g.* allantoin), etc.

The lead acetate precipitate (A) was found to contain only ·85 per cent. of nitrogen, evidently as protein. It gave the xanthoproteic test, but no tryptophane or murexide reactions, and was not further examined.

Examination of the mercury precipitate (C). This was suspended in water and decomposed with sulphuretted hydrogen, the solution was then concentrated and left to crystallise. No crystals could, however, be obtained, but only a syrup, with a strong glue-like smell. It readily yielded brightly coloured copper salts with copper hydroxide, but all attempts to resolve it into its constituents failed. No tyrosin reaction with Millon's solution was obtained. Ferric chloride gave a red colour which indicates the presence of **glycocol** (amido-acetic acid) and nitrous acid produced a mixture from which lactic acid was isolated, indicating that **alanine** (and amido-propionic acid) is present. More definite proof could not, however, be obtained.

Examination of the phosphotungstic acid precipitate. For the preliminary tests Kossel's method was used[1]. The precipitate was suspended in water in a mortar, and ground with successive quantities of barium hydrate till the liquid had an alkaline reaction. The barium combines with the phosphotungstic acid, and liberates the bases which pass into solution. After filtering, carbon dioxide is passed through the liquid till the dissolved baryta is all precipitated, it is filtered and the filtrate saturated with carbon dioxide. Mercuric chloride is then added till the mixture becomes acid. A precipitate was produced indicating the probable presence of histidine (D).

This was filtered, the mercury in the filtrate was removed by sulphuretted hydrogen, and after again filtering, the chlorine in the filtrate was removed by silver sulphate. The filtrate, which now

[1] *Zeitschrift für Physiolog. Chemie* (Hoppe-Seyler), 1896, 22, 177 and 1898, 26, 586.

contains the bases as sulphates, was warmed, and treated with silver sulphate till a small test portion gave a brown precipitate with baryta. Baryta was then added to the bulk of the liquid till precipitation was complete. The precipitate would contain arginin, if present (E).

The filtrate is treated with CO_2 to remove barium, and with HCl to remove silver. An alcoholic solution of picric acid was then added taking care to avoid excess, this gave a beautifully crystalline precipitate indicating the presence of lysin (F).

The filtrate was treated with hydrochloric acid and extracted with ether to remove picric acid. Phosphotungstic acid was added and produced a copious precipitate, showing that other bases were present. These were liberated, as before, by treatment with baryta and subsequent treatment with carbonic acid. They were then converted into their hydrochlorides and concentrated.

Gold chloride was now added. A precipitate was produced indicating the presence of other bases.

The mercuric chloride precipitate D was decomposed by suspending in water and treating with a current of sulphuretted hydrogen. On evaporation a few crystals were obtained which gave a magnificent cherry red colour with an alkaline solution of diazobenzène sulphanilic acid characteristic of imido-azol compounds having the hydrogen in the (7) position not replaced. Histidin shows this reaction.

The silver precipitate was decomposed by sulphuretted hydrogen and the filtrate concentrated. The solution gave no precipitate with phosphotungstic acid, and it is therefore probable that **arginin** is **absent.**

The picrate was decomposed with hydrochloric acid and extracted with ether to remove picric acid. On evaporation of the aqueous layer crystals of a hydrochloride soluble in methyl alcohol were obtained. This appeared to be **lysin** hydrochloride.

The original solution of the bases was also found to give the reactions of the purin bases. Ammoniacal solution of silver nitrate and a mixture of sodium bisulphite and copper sulphate both gave precipitates; a yellow residue was also obtained on evaporation with nitric acid, which turned purple when treated with caustic soda.

The method adopted for the isolation of the bases was as follows:

The phosphotungstic acid precipitate was, as before, ground up with baryta, excess of which was subsequently removed. The solution containing the free bases was just acidified with nitric acid and concentrated; it was then made distinctly acid with nitric acid and treated with silver nitrate. A white precipitate was formed (E).

To the filtrate ammonia was added till the solution was barely alkaline. A white precipitate (F) was obtained.

E gave the reactions of the purin bases, but it contained 64·4 per cent. of silver, which does not correspond with any of their ordinary silver salts. The amount obtained was too small to allow of further examination.

F proved to be silver histidin salt obtained by Hedin, $C_6H_7Ag_2N_3O_2$, H_2O,

Silver found = 55·79, theor. 55·82.

Histidin is therefore present.

The filtrate from F was now treated with phosphotungstic acid and the bases once more obtained in the free state. They were strongly alkaline and had an exceedingly disagreeable odour characteristic of the amines. The lysin was precipitated as picrate, the picric acid was removed from the solution and the bases were converted into hydrochlorides and evaporated to dryness; the residue was extracted with absolute alcohol.

Examination of the hydrochlorides soluble in absolute alcohol. This might contain cholin, cadaverin, putrescin, etc. Addition of alcoholic platinum chloride produced a precipitate which on purification and ignition yielded 37·72 per cent. of platinum. The precipitate therefore appears to be the platinum salt of **pentamethylene diamine** (cadaverin) which contains 38·06 per cent. of platinum. No other platinum salt appeared to be mixed with it, the separate fractions all containing practically the same amount of platinum.

Examination of the hydrochlorides insoluble in absolute alcohol. This was dissolved in a little water and tested with gold chloride. A precipitate was obtained, which however was quite different in character from the ordinary betain compound. The hydrochloride was found to give a precipitate with mercuric nitrate and to form a copper salt, it was therefore a diamino acid, presumably **αδ diaminovaleric acid** (ornithin) since it also gave a precipitate with mercuric chloride. The gold salt was prepared and analysed,

Au found = 38·88, calculated for $C_5H_{12}N_2O_2 . 2HCl . AuCl_3$ 38·76.

The filtrate still contained bases, but their nature has not been ascertained. A reducing body is present, since metallic gold was deposited from the solution on standing. Traces of betain were found on one occasion to be present.

The purin bases. Owing to the small quantity present in the juice it was necessary to examine the solid part of the silage. This was extracted

with water to remove easily soluble bodies and then with dilute nitric acid. The extract gave the reactions characteristic of purin bases—precipitates with ammoniacal silver nitrate, with copper sulphate and sodium bisulphate, and the usual colour reactions on evaporating with nitric acid and moistening with caustic soda, but the amount obtained was too small for complete examination. On treating with mercuric nitrate a precipitate was obtained from which a small quantity of base was isolated which gave the silver and copper precipitates, gave a red colour with diazobenzene sulphanilic acid, sublimed on heating and gave a crystalline gold chloride. These are also the reactions shown by **6-amino-purin (adenin)** which therefore appears to be present.

Au found = 37·0, calculated for $C_5H_5N_5 . 2HCl . AuCl_3 . H_2O$ 37·2.

Since, however, adenin does not give the xanthin colour test it is clear that another purin base is also present.

After the nitric acid was removed from the silage ammonia was added and a dark-coloured, fluorescent solution was obtained. This also gave the reactions characteristic of the purin bases, but it also contained a certain amount of humus, which proved so troublesome that further examination was not made.

The following substances were tested for, but not found:—allantoin, kreatin, kreatinin.

Conclusions.

1. The main groups of compounds found in maize silage are fatty acids, hydroxy- acids, amino acids, basic diamino acids, purin bases, and other bases, besides the ordinary constituents of the plant cell, the celluloses, protein, etc. The non-nitrogenous acids are not found in maize at the time of cutting, and the nitrogenous acids, though they are found, occur to a smaller extent than in silage.

2. The characteristic silage changes are the disappearance of sugar, of some less resistant celluloses, and of part of the protein, and the formation of the bodies enumerated above.

3. Three agents appear to be involved in making silage, the living maize cell, the enzymes, and micro-organisms. It is considered that the two former bring about the primary and essential changes, the latter only secondary and non-essential changes.

4. The formation of acetic and butyric acids appears to be a respiration effect, and comes about when the living cell is deprived of oxygen. Sugar disappears during the process.

The decomposition of the protein and nucleo-protein is effected by enzymes present at the time of cutting the maize, which can go on acting in the silo even after the cell is dead. Characteristic products of protein-hydrolysis were identified in the silage.

These are regarded as the primary and essential changes.

5. Bacteria are, however, always present, and attack the less resistant celluloses, the products of protein-hydrolysis, and no doubt other substances as well, but not the resistant fibre. Typical products of bacterial activity were found—formic acid, higher fatty acids, humus and amines.

6. The growth of mould is inhibited except at the surface layer where air gets in. Here the changes are fundamentally different; there is no development of acetic or butyric acids, the mass is alkaline, non-protein material already existing in the maize is converted into protein, and there is also a loss of nitrogen.

STUDIES ON GERMINATION AND PLANT-GROWTH.

By SPENCER UMFREVILLE PICKERING, M.A., F.R.S.

EXPERIMENTS which have been in progress at the Woburn Experimental Fruit Farm on the effect of grass on trees, have led to the conclusion that this effect cannot be attributed to any such simple causes as root-competition, increased evaporation, or differences in temperature or aëration of the soil, but must be due to some poisoning action exerted by the grass. Whether, however, such action is a direct result of the growth of the grass, or an indirect one, operating through the medium of soil-bacteria, there is, at present, no evidence to prove.

Behaviour of trees in heated soil.

As an initial experiment on this aspect of the case, 26 two-year old apple trees were planted in pots under various conditions, including the use of heated soil. The trees were planted in February, and were kept out in the open after planting; three of them were in soil from Ridgmont (see p. 427) which had been heated for two hours at 82°, and six in soil heated to about 200°, the soil in three out of these six having been previously powdered and sifted. The sterilisation of the soil at 82° would, of course, be only partial, and in all cases there would be opportunity for re-inoculation to occur. The pots were kept constant in weight by additions of water every other day, and no artificial nourishment was given.

One of the nine trees in heated soil almost died, but the other eight soon became conspicuous for the extra growth which they were making, as well as for the darker colour of their leaves, and these eventually remained on the trees two or three weeks after the normal date of falling. Below are given the length of new wood formed, the total weight of the leaves, the percentage of nitrogen in the dried leaves, and the total dry matter and nitrogen in the leaf-crop: the actual quantities, where given, are in

grams; the relative values are obtained by comparison with those given by the trees in unheated soil. For the nitrogen determinations I am indebted to Dr E. J. Russell, who had them made in the Lawes' Laboratory by the kind permission of Mr A. D. Hall.

TABLE I. *Apple trees (King of the Pippins) grown in heated and unheated soil.*

Soil	Length of new wood. Relative	Weight of leaf-crop. Relative	p.c. nitrogen in leaves. Actual; Rel.	Dry matter in leaf-crop. Actual; Rel.	Nitrogen in leaf-crop. Actual; Rel.
Unheated	100, 107, 92 } 100	90, 100, 110 } 100	1·735=100	21·6=100	·375=100
Heated to 82°.....	88, 190 } 139	109, 172 } 141	1·826=105	31·1=144	·576=151
Heated to 200° ...	190, 102, 176 } 163	180, 126, 137 } 148	2·036=117	32·8=152	·667=178
Ground and heated to 200°	144, 90, 151 } 128	190, 108, 112 } 137	2·446=141	26·0=120	·635=170

The trees in soil which had been heated show a much greater development both of wood and leaf than those in unheated soil, also, the leaves in the former case are the richer in nitrogen, so that the total nitrogen assimilated shows an excess of as much as 50 to 80 per cent. All the properties examined show that the heating at 200° has been more beneficial than that at 82°, but, where the soil was ground as well as heated, the indications are less uniform, the leaves being richer in nitrogen than in any other case, but the dry matter in them, and also the length of wood formed, being comparatively low. No doubt the beneficial effect of heating has been facilitated by the grinding, but the fine state of division of the soil has interfered with the proper functioning of the roots through lack of free aëration. There is much less uniformity, it may be observed, between the duplicates in heated soil than between those in the unheated soil: a similar lack of uniformity in the behaviour of heated soils will be noticed in other cases.

These results agree with those of Darbishire and Russell recorded in this Journal[1]. These workers had previously shown[2] that the rate at which soil absorbs oxygen is greatly reduced when the soil is nearly

[1] Vol. II. p. 305. [2] Vol. I. p. 260.

sterilised by heating to 120°, but when heated to only 95°, its absorptive power is considerably increased: assuming that oxygen absorption and bacterial contents go hand in hand, this would accord with Hiltner and Störmer's observation that heating to 95°, though it initially reduces the number of bacteria in the soil by 75 per cent., results eventually in an enormous increase in the numbers present. Oxygen absorption, according to Darbishire and Russell, is an index of the fertility of a soil, and, in accordance with this, they found that a number of different plants, other than Leguminosæ, showed an increase in growth ranging from 33 to 380 per cent. when grown in soil heated to 90—95°. The leaves were found to be relatively rich in nitrogen, and the total amount of this element removed from the heated soil by the plant was sometimes three times as much as that removed from unheated soil. It must be remarked, however, that the beneficial effect of heating was found to be increased when a temperature of 120° was employed, and as such a temperature greatly diminishes the oxygen absorption, and nearly, or entirely, sterilises the soil, this fact would appear to exclude either oxygen absorption or bacterial conditions from being the main factors in producing the increased growth. On the other hand, partial sterilisation with antiseptics produced results similar to, though considerably smaller than, those produced by heating to 90—95°, and here, it was argued, the results must depend, at least partially, on the altered bacterial conditions of the soil.

The present experiments add apple trees to the list of plants showing increased growth and nitrogen-assimilation in heated soils. They, however, behaved very unexpectedly in another respect, for they did not start into growth till long after the trees in unheated soil. The latter, of which there were seventeen, were planted under a variety of conditions, but they started at the same time, or within one day of each other. Of the nine trees in heated soil, however, only two (two of those in unsifted earth heated to 200°) started on this date, another did not start till late in the summer, and almost died, whilst the remaining six were quite fourteen days behind those in unheated soil. This delay was attributed at first to the altered bacterial conditions of the soil; and the fact that two of the trees were exceptional in their behaviour, lent colour to such an explanation; for chance re-inoculation of the soil was very possible, whereas chance alteration in its chemical character was hardly so.

An examination of the effect of soil conditions on the starting of trees into activity promised to be such a lengthy task, that it seemed

advisable to attempt to obtain some preliminary light on the subject by first examining a phenomenon of an allied character, namely, the germination of seeds.

Preliminary experiments on germination.

In the first series of experiments, the seeds of *Lolium perenne* were sown in Harpenden earth or in "burnt" sand, which had been heated in an air-bath to various temperatures for 2 hours. The seeds were partially sterilised in carbon disulphide vapour, and were sown at a depth of $\frac{1}{8}$ inch from the surface of the soil or sand, which was placed in bottles through which a current of filtered air was occasionally drawn. All possible precautions were taken to render the conditions aseptic. Ten seeds were sown in each bottle, and each experiment was made in duplicate: these were all very concordant, and the mean results only are given in Table II. The relative values are those compared with the results in unheated earth.

TABLE II. *Lolium perenne in earth and sand.*

Treatment	Earth			Sand		
	Germination, %	Incubation, days	Relative weight of plants	Germination, %	Incubation, days	Relative weight of plants
Unheated	65=100	11=100	100	65=100	14=130	65
Heated at 60°	35= 54	19=180	3·3	—	—	—
,, 80°	5= 8	25=231	0·8	—	—	—
,, 100°	5= 8	69=639	0·9	0	∞	0
,, 150°	0	∞	0	—	—	—
,, 250°	0	∞	0	45=69	15=139	2·6
Heated at 250° and reinoculated	55= 85	23=213	10	20=46	16=143	30
Treated with CS_2	80=123	12=107	66	—	—	—

It is evident that the heating of the soil has been very detrimental to germination: even when the temperature had been raised to only 60°, the number of seeds germinating was reduced to nearly half, and the time occupied in the germination of these was nearly doubled; whilst heating the soil to 150° prevented germination altogether. Re-inoculation of heated soil, by adding to it a 10 per cent. soil extract instead of water, has almost restored it to the con-

dition of unheated soil: on the other hand, partial sterilisation by carbon disulphide vapour has had little or no effect. The results in sand are similar to those in earth, though less regular, heating to 100° having prevented germination altogether, although the heating to 250° has had comparatively little effect on the germination percentage, and practically none on the incubation period: inoculation with earth extract has had little effect on the germination. The analogy between the behaviour in heated sand and heated earth is greater when the weight of the seedlings is considered, the heating in both cases having resulted in a remarkable diminution of the growth of the plants, amounting, in the case of earth, to twenty-nine thirtieths at 60°, and to more than ninety-nine hundredths at 80° and 100°. Inoculation with soil extract has also produced analogous results with earth and sand.

In two respects these results appear to be in opposition to those of Darbishire and Russell. These chemists found no effect to be produced on the germination of the seeds by heating the soil[1], and they also found, as already mentioned, that the heating resulted in increased growth. Their experiments, however, were conducted under very different conditions from the present ones, and it will be shown below that the Wye soil used by them exhibits the same retarding effect on germination when heated, as does the Harpenden soil. As to the growth of the plants, the discrepancy may be apparent only: the present results can hardly be accepted as showing what the real behaviour of the plant would be, for, when thus grown in closed bottles, the conditions are quite abnormal, and the plants never reach maturity; in fact, the growth exhibited may, perhaps, be regarded more as extended germination, than as plant-growth. It may be remarked, however, that the roots must have been functioning to a considerable extent, for the ash of the plants grown in unheated soil was 120 times that of the seeds producing them, and, even in the unheated sand, the plant-ash was 100 times that of the seed-ash.

The fact that heating sand had a similar inhibitory effect to that of heating soil, naturally led at first to the conclusion that the question was one of bacterial, and not of chemical, change[2], and it was with this idea that the investigation was pursued.

Method of experimentation.

The further experiments were carried out in sporulating dishes in an incubator at about 23°. Each dish held 15 grams of soil, and

[1] *Loc. cit.* p. 322, and *Nature*, July 4, 1907.

[2] *Nature*, June 6, and July 4.

10 seeds were either just buried below the surface of the soil, or, in the later experiments, were simply pressed into it by a platinum disk. The water contents were made up to 23 per cent.

In every case the experiments were made in duplicate, but the means only will be given. The probable error of these, in the case of the times of incubation entered in Table III, as determined by the differences between duplicates, varies according to the temperature to which the soil has been heated, and is:—

Unheated	*Heated to*				
	60°	*80°*	*100°*	*125°*	*150°*
5·4	6·8	8·4	20·7	16·8	39·7 units.
or 5·4	6·2	6·8	14·7	9·1	16·7 per cent.

To obtain uniform results it was found essential that each series of experiments with one kind of seed in soil heated to different temperatures should be made at the same time.

Seed sterilisation.

Carbon disulphide chloroform and ether were each found to be inefficient for sterilising the seeds: formalin—used by driving the vapour from a boiling solution over the seeds, and then leaving these in a tube plugged with wool till all smell had disappeared—was generally effectual, but no means were found for regulating the treatment. Mercuric chloride was finally adopted. The seeds were first treated with ether and evacuated, washed with alcohol, and next with water, and then soaked for 20 minutes in a 0·25 per cent. solution of the chloride, being finally washed thoroughly with sterilised water. There did not appear to be much difference in the sterilising action of a mercuric chloride solution of this strength, and one of half or double the strength; but a 0·5 per cent. solution acting for 40 minutes proved fatal to the seeds. The time occupied in preparing and sowing twenty or thirty dishes with seeds was considerable, and chance re-inoculation could not always be avoided; but some of the seeds were always tested in agar-agar, and occasionally the sterilisation was found to be imperfect, about one seed out of ten giving rise to mould: the mould, however, often appeared only after the lapse of 8 to 15 days, indicating that the germs must have been actually inside the seed. In the following tables, where the test showed this imperfect sterilisation, "sterilised" is placed in brackets, "(St.)"

Germination in agar-agar was obtained with perfectly sterilised seeds of *Lolium italicum*, *Lolium perenne*, clover, spinach and, in one case,

mustard: to these may be added turnips, which were germinated by Dixon under aseptic conditions[1].

Treatment with sterilising agents increases the time required for germination, and (generally) reduces the percentage of seeds germinating, as will be seen by the following values:

Treatment of seeds	Mustard		Rye grass	
	% germination	Time, days	% germination	Time, days
Water	100	2·0	88	4·4
Ether and CS_2	100	2·2	91	4·8
Ditto and $HgCl_2$ 1 : 800...	90	2·4	68	4·5
,, ,, 1 : 400...	90	2·6	91	6·9
,, ,, 1 : 200...	100	2·8	68	6·3

Wheat, rye, mustard, clover, Lolium italicum, Lolium perenne, Dactylis glomerata, and Poa annua. Mean.

	In earth		In sand	
	% germination	Time, relative values	% germination	Time, relative values
Water	100	100	100	100
$HgCl_2$, 1 : 400	80	160	81	221

The soil used.

This was the same as that used in the preliminary experiments, but 4 per cent. of pure calcium carbonate was added to it, and the heating was conducted (for two hours) in closed vessels in an autoclave. The water contents at the time of heating were A, 18·03 p. c., B, 9·28 p. c. and C, 13·5 p. c., in the case of the different batches of soil which had to be prepared.

The water was always made up to 23 per cent. after the seeds were sown; but the results were found to be independent of the amount of water within somewhat wide limits: thus, the behaviour of clover and mustard in soils heated to 60°, 125° and 150° was found to be practically identical, whether these contained 18 or 28 per cent. of water, the average germination percentages being 102 : 100 in the two cases, and the incubation times 104 : 100. As the loss of water during the experiments was found to be only 0·25 per cent. *per diem*, irregularities in the rate of this loss could have no appreciable effect on the results.

The soils heated to 125° and 150° were found to be quite sterile: that heated to 100° decomposed broth much less readily than the soils heated to lower temperatures, and showed a comparative absence of putrefactive bacteria: the soil heated to 80° was less active than that heated to 60°, and this, again, was less active than the unheated soil.

[1] *Trans. Roy. Soc. Dublin*, v (11), p 1.

Results obtained.

Table III contains the results from 21 series of experiments. The first eleven were made with soil sample A, and the rest with B. Six of the first eight were made with seeds which had been sterilised, though, in some cases, not perfectly so; the others are mainly with unsterilised seeds. The last six series consist of three pairs of results, in which sterilised and unsterilised seeds were used, these series being made at the same time. The actual percentage of seeds germinating, and the average time in days required for the germination, are given as regards the unheated soil only: as regards the heated soils, the relative values, compared with those in the unheated soil expressed as 100, are alone given.

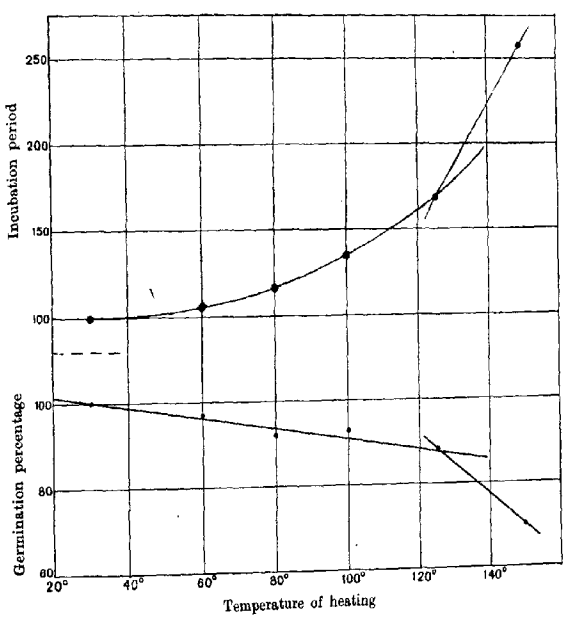
TABLE III. *Percentage of germination and time of incubation of seeds in soil heated to various temperatures.*

Seeds	Percentage germination						Time of incubation					
	Unheated soil, actual	Relative values					Unheated soil, actual	Relative values				
		60°	80°	100°	125°	150°		60°	80°	100°	125°	150°
Mustard (St.)	100	100	100	95	75	40	2·4	94	99	107	270	574
Lol. ital. St.	60	100	150	125	108	75	6·7	99	93	134	167	206
Rye (St.)	50	170	120	130	120	100	2·0	145	158	235	388	245
Clover St.	90	111	106	95	61	17	2·1	102	117	180	207	402
Lol. peren. St.	15	(167	300	367	233	233)	7·0	107	129	136	100	146
Dact. glom.	20	175	50	100	75	25	15·0	64	113	90	123	80
Poa annua	55	9	36	82	27	56	5·6	101	171	124	149	261
Wheat (St.)	45	100	78	78	78	22	5·2	73	126	125	125	144
Mustard	100	—	100	—	95	80	2·4	—	86	—	194	557
Clover	95	—	100	—	90	63	2·1	—	162	—	169	310
Rye	85	—	100	—	94	106	2·0	—	106	—	108	130
Clover	100	100	100	95	80	85	1·8	103	117	133	150	278
Lol. ital.	65	77	115	85	145	77	3·6	90	136	133	146	428
Rye	85	118	106	118	112	106	2·5	134	86	94	146	174
Wheat	100	100	95	100	100	95	4·9	94	87	99	106	142
Fest. prat. (St.)	95	84	95	100	84	95	4·1	115	122	143	149	207
" "	90	106	94	100	100	100	3·6	110	103	117	143	147
Spinach St.	35	72	130	126	163	140	5·3	72	130	126	163	140
"	100	100	100	100	85	95	4·5	107	107	151	169	168
Fest. ov. (St.)	35	57	29	43	29	14	9·3	108	119	132	141	173
" "	35	71	29	14	43	0	8·0	144	106	125	137	—
Mean (relative)	100	97	92	93	88	70	100	106	117	135	169	260

In spite of numerous irregularities with individual series, it is clear that, on the whole, the time of incubation increases considerably with an increase in the temperature to which the soil has been heated: at the same time the percentage of seeds germinating decreases with this heating, though the irregularities in this case are considerable, and there are several instances which indicate that heating the soil to the lower temperatures (60° or 80°) has actually increased the germination.

The general features of the results are evidently the same with both samples of soil, and the differences observed in different series appear to be due mainly to the differences in the behaviour of the seeds, some (*e.g.* mustard) being evidently more affected by the heating of the soil than others (*e.g.* rye). The last six series, as well as others, show also that the results are practically the same whether the seeds have been sterilised or not: the mean differences in the times of incubation of the sterilised and unsterilised seeds in these series being:

60°	*80°*	*100°*	*125°*	*150°*
+22	−18	−3	−5	−16



The values in the table, being entered in chronological order, afford evidence that the heated soil preserves its deleterious properties on keeping, at any rate at a winter temperature; for in many of the above cases several months had elapsed between the making of the corresponding series.

In spite of irregularities in the individual series, the mean results are remarkably regular, and, when plotted out, produce the figures shown in the diagram on page 419. (The germination percentages in the case of *Lolium perenne* have been omitted from the mean, as the total germination was only 15 per cent., and the results were proportionately uncertain.) These, in the case of the germination percentages, lie sensibly on a straight line up to 125°, and the incubation times lie very closely on a bent lath curve up to this same temperature. It would, however, require fuller data to warrant the conclusion that the apparent alteration in the action at about 125° is a reality. The "unheated" soil was probably heated to about 30° during its drying in air, and the results with it have been entered for this temperature.

Results with sand.

Before discussing these results, some similar experiments with sand may be described. The sample of sand used in the preliminary experiments had been exhausted, and two other samples were used in the experiments entered in Table IV. The first was a mixture of "burnt"

TABLE IV. *Percentage of germination and time of incubation of seeds in sand heated to various temperatures. Relative values, those in unheated sand being* 100.

Seeds	Percentage germination					Time of incubation				
	60°	80°	100°	125°	150°	60°	80°	100°	125°	150°
Mustard (St.)	100	95	100	95	100	110	93	90	71	88
Lolium italicum St.	94	94	88	94	81	75	79	71	63	93
Rye (St.)	127	155	127	130	127	96	109	81	85	76
Dactylis glomerata	(0	33	167	33	67)	—	100	101	100	123
Mustard (St.)	—	—	100	—	100	—	—	105	—	110
Clover St.	—	—	90	—	105	—	—	108	—	81
Lolium perenne St.	—	—	80	—	100	—	—	105	—	87
Poa annua	—	—	110	—	90	—	—	101	—	101
Mean	107	115	99	106	100	94	95	100	80	95

sand with coarse and fine Leighton sand; the other (used in the last four series entered) was some fine Leighton sand which had been buried in earth for twelve months, so that it should become impregnated as far as possible with soil bacteria.

The results as will be seen are purely negative. Both the means of the germination values (from which those with *Dactylis glomerata* are omitted, owing to the small number of seeds which started), and the means of the incubation times, are all very close to 100—the value in unheated sand—and show no tendency to vary in any definite direction. Whatever the explanation, therefore, of the results with sand in the preliminary experiments may be, it is clear that those results were due to some peculiarity of the particular sample of sand used: no doubt, it must have been accidentally contaminated with sufficient organic matter to make it behave like ordinary soil (see p. 423).

The bacterial explanation.

Summarising the evidence for or against the view that bacterial conditions are factors in the germination of seeds, we have:

First, the fact that many seeds will germinate under aseptic conditions in a sterile medium (p. 417), and that, therefore, bacteria cannot always be necessary factors in the case. Secondly, taking the results with heated soils, the effect of heating on germination is evident at temperatures as low as 60°, probably, even 30°, and is also progressive with the temperature, certainly up to 150°, whereas the bacterial conditions of the soil cease to be affected by any heating above 125°, all bacteria having been killed at that temperature. Thirdly, sand, even when thoroughly inoculated with soil bacteria, behaves in the same way towards germination whether it is heated or not.

Lastly, some experiments were made to see whether the results would be affected by re-inoculating previously sterilised seeds. Seeds of rye-grass and mustard were first sterilised with mercuric chloride, and then half of them were soaked for an hour in a decoction of similar, but unsterilised, seeds; the other half being soaked in sterile water for a like time. Each lot of seeds was then sown, both in unheated earth and in heated sand. The incubation periods of the re-inoculated seeds as compared with those of the sterile seeds (= 100) were

Rye grass	In earth	69
	In sand	114
Mustard	In earth	104
	In sand	100
	Mean	97

thus showing that the re-inoculation had had no effect.

The chemical explanation.

A bacterial explanation of the facts being thus put out of court, a chemical explanation must naturally be sought. That soil on being heated, even to 100°, undergoes chemical change, is well known, and this change, as evidenced by the increasing depth of colour of the aqueous extract, increases at higher temperatures. No acid, however, appears to be produced, at any rate when the soil is heated in a moderately moist condition, and with an excess of chalk in closed vessels, as was the case in the present work.

The aqueous extracts of the soils were investigated as regards the amount of organic and mineral matter contained in them, and also as regards their nitrogen contents. The difference between the weights of the residue dried at 100° and after ignition is entered as organic matter, but it is probable—from the results obtained with some soil which had been heated to redness (p. 425)—that it embraces also a certain amount of constitutional water retained at 100°. For the nitrogen determinations, I am again indebted to Dr E. J. Russell. The results are given in Table V, soil sample B being used for the determination of extract, and sample C for that of the nitrogen.

TABLE V. *Soluble matter in soils heated to different temperatures.*

Temperature of heating	Soluble matter				M/I	O/I	N/I
	Mineral	Organic	Nitrogen	Organic %			
Unheated	·266	·315	·00402	54	—	—	—
60°	·254	·340	·00397	57	—	—	—
80°	—	—	·00425	—	—	—	—
100°	·311	·413	·00796	57	·0013	·0028	·00011
125°	·312	·458	·01553	59	·0007	·0021	·00017
150°	·423	·845	·02921	67	·0010	·0033	·00016

The heating increases the soluble mineral matter, and still more so the soluble organic matter, especially above 125°, the ratio of the two being given in the column headed "Organic, %." The nitrogen contents, also, increase with the temperature, the seeming irregularity at 60° being within the limits of experimental error.

To ascertain whether any direct connexion can be traced between the composition of the soil and the germination results, the increase in the soluble soil constituents has been divided by the increase in the times of incubation as given in Table III. The values obtained by

treating the mineral, organic and nitrogen contents in this way are given in the columns headed M/I, O/I and N/I respectively. In the first two of these, an accumulated error of 2 milligrams in the weighings (independent of any error in the incubation values) would make a difference of ·0002 in the value at 150°, ·0007 in that at 100° and ·004 in that at 60°: this precludes the possibility of attaching any importance to the values at 60° in either case, and, indeed, to any of the values for M/I: it is impossible, therefore, to say how far the incubation results are dependent on the increase in the soluble mineral contents of the soil. As regards O/I the values from 100° to 150° are certainly constant within the limits of experimental error. With the nitrogen values, the experimental error would probably make a difference of at least ·00004 in the value at 100°, and ·000015 in that at 150°, so that here, again, we have a constant well within the limits of experimental error: at 80° and 60° the experimental error is greater than the differences to be measured.

These results, therefore, justify the conclusion that the effect on the germination of seeds produced by heating a soil is directly proportional to the amount of some organic and nitrogenous compound formed during the heating, and is, therefore, in all probability, a direct consequence of it: whether alteration in the mineral constituents of the soil has also an effect the results so far do not settle, but conclusive evidence on this point will be given below.

Heating soils under various conditions.

Some determinations were made to ascertain how far the amount of this organic substance formed on heating a soil was affected by the proportion of water present during the heating. Three samples of the same lot of soil, dried to different extents by exposure to air, were heated

TABLE VI. *Composition and behaviour of soil heated to* 140° *with different amounts of water.*

Water contents of soil	Contents of extract		Relative times of incubation			
	Mineral	Organic	Mustard	Clover	*Lolium perenne*	Mean
14	·345	·545	88	114	116	106
7	·190	·463	83	104	95	95
2·7	·187	·475	100	100	100	100

at the same time in the autoclave at 140° for 2 hours. The composition of their aqueous extracts (Table VI) indicates that the two samples where there was least water were almost identical, whilst that with the greatest amount gave distinctly more soluble organic matter, and nearly double the amount of soluble mineral matter. The germination results obtained in these soils do not in any of the three cases investigated follow the values for the soluble mineral matter, but the mean results do follow those for the soluble organic matter, though the differences are small. Similar results with soils heated at 150° with different amounts of water will be found in Table VII.

TABLE VII. *Composition and behaviour of soils heated to high temperatures.*

Series I. Mean of results with mustard, clover, *Festuca pratensis*, rye, wheat and *Lolium italicum*.
Series II. Mean of results with mustard and *Festuca pratensis*.
Series III, IV and V with mustard.

Treatment of soil	Contents of extract		Incubation times of seeds				
	Inorganic	Organic	Series I	Series II	Series III	Series IV	Series V
Unheated	(·266)	(·315)	—	55	22	(30)	(30)
150° moist	(·423)	(·845)	—	—	—	167	172
150° dry	·256	·616	100	100	100	100	100
200° ,,	·339	·634	118	—	—	—	—
250° ,,	·267	·493	103	—	—	—	—
350° ,,	·287	·235	—	81	40	—	—
Redness	·210	·070	—	(63) (61)	23	—	—

In some other experiments the question of the effect of the length of time for which the soil was heated, was investigated. Some of the same sample of soil (containing 9 per cent. of water) was heated at 100° for 2, 6 and 12 hours respectively; and the relative incubation periods obtained with these samples were:

	Clover	*Festuca pratensis*	Mean
Heated 2 hrs.....	100	100	100
,, 6 ,,	90	136	113
,, 12 ,,	105	92	99

from which it appears that they all behaved in a similar manner within the limits of experimental error.

If the altered behaviour of soils is due to the organic substances rendered soluble by the heating, a temperature sufficiently high to destroy these substances should result in restoring the soil to its original germination capacity.

For heating above 150°, an air-bath had to be used: this involved a total loss of the water present. The results with soils thus heated are given in Table VII, in which are also inserted in brackets, for the sake of comparison, the results with moist soils heated at 150° in the autoclave, and also with unheated soils, as given in previous tables. The heating (soil C, being used) was for 2 hours at the temperatures named, except with the sample heated to low redness in an open dish, where 1 hour only was allowed. What is entered as soluble "organic" matter in this sample must be chiefly constitutional water retained by the inorganic residue at 100° (see p. 422).

The germination experiments were, unfortunately, not well devised, and several series have had to be pieced together; the results with soil heated to 150° have been taken as the common standard of comparison. The first series, with six different seeds, gives 200° as the temperature of heating which produces the maximum retarding effect, and this agrees with the temperature at which the organic matter rendered soluble is greatest. With higher temperatures, both the retardation and organic matter decrease (series II and III), till, at a red heat, the latter becomes nil, and the incubation time of the seeds is the same, or very nearly the same, as in unheated soil. These results, therefore, afford further strong evidence that it is the organic matter rendered soluble by the heating which is the inhibitory agent as regards germination: they cannot be explained by the variations in the soluble inorganic matter.

The difference in the composition of the soils heated to 150° with and without drying, and the different effect of them on seeds, as shown by the 4th and 5th series, afford additional evidence in the matter, and supplement the results already given in Table VI.

Behaviour of the inhibitory substance in heated soils.

That the extract of a soil which has been heated will impart its inhibitory properties to a soil which has not been heated, is shown by the results in Table VIII, wherein unheated soil was treated with various quantities of an extract obtained by digesting soil which had been heated to 150° with an equal weight of water. Moreover, the retardation of the germination appears to be directly proportional to percentage of inhibitory matter present in the water contained by the soil. The calculated values

are based on this supposition, and they agree within experimental error with the values found in the case of mustard: the differences in the case of *Festuca* are too small to lead to any conclusions.

TABLE VIII. *Effect of treating soil with extract of heated soil.*

Soil used	Time of incubation			
	Mustard		*Festuca pratensis*	
	Found	Calculated	Found	Calculated
15 grs. unheated + 3·5 c.c. water.....	100	—	100	—
15 grs. unheated + 3·5 c.c. extract ...	129	123	103	102
15 grs. unheated + 7 c.c. extract.....	133	128	105	103
15 grs. heated + 3·5 c.c. water.....	271	—	110	—

On the other hand, unheated soil does not appear to contain anything which will appreciably counteract the inhibitory action of heated soil. Soil which had been heated to 150° was digested for 5 days in the incubator at 23° with extract of unheated soil—this extract being de-

TABLE IX. *Results with soil digested with water, etc. before sowing.*

Soil used		Treatment of soil					Seeds used
Heated to	Water %	Not digested	Digested with			Unheated soil	
			Extract	Lime-water	Water		
150°	26	100	72	69	66	30	Clover
150°	26	100	97	98	104	57	*Festuca*
—	—	*100*	*85*	*84*	*85*	*44*	*Mean*
150°	23	100	—	—	79	28	Mustard
150°	17	100	—	—	116	30	Clover
150°	26	100	—	—	81 (99)		
125°	19	100	—	—	72	56	
125°	28	100	—	—	136 (104)		
60°	20	100	—	—	92	102	
60°	29	100	—	—	86 (89)		

rived from a weight of soil equal to one-sixth of the heated soil in the dishes—and though the incubation period of seeds then sown in it was found to be reduced from 100 to 85 (Table IX), a precisely similar

reduction occurred when lime-water or pure water was added, instead of soil-extract. Had the inhibitory action been entirely destroyed, the incubation period would have been reduced to 44 in these cases. Further experiments on the digestion of other heated soils with various proportions of water are given in the table, and they lead to very variable results; in most cases some reduction of the incubation period has been produced, but the average of the last six entries gives this reduction as only 3 per cent. Probably the destruction of the inhibitory substance is due to a process of oxidation, which is affected by the minutiae of the circumstances attending each different case.

That no appreciable destruction of the inhibitory substance occurs when the soil is kept for several months at low temperatures, and in a moderately dry condition, has already been mentioned (p. 420).

Behaviour of different soils.

It was of importance to ascertain how far the phenomena exhibited by the Harpenden soil were common to other soils. Three soils were selected for the purpose: one from Ridgmont, Beds., which is a fairly fertile soil on the Oxford clay; another from the chalk formation at Wye, Kent—this being the same soil as that used by Darbishire and Russell in their work—; and a third from Millbrook, Beds., where the soil is excessively sandy and poor. The soils were all taken from land under cultivation, as also was the Harpenden soil, although in that case the land had consisted of old pasture previous to 1893. Analyses of these soils are given in Table X, though 4 per cent. of chalk was added

TABLE X. *Analyses of the soils used.*

	Harpenden	Ridgmont	Wye	Millbrook
Nitrogen	0·25	0·28	0·17	0·10
Water and organic matter	6·94	6·30	3·94	2·75
K_2O	0·27	0·60	0·35	0·18
CaO	0·43	1·03	5·6	0·35
Iron oxide and alumina	10·19	—	7·94	5·66
P_2O_5	0·114	0·22	0·143	0·15
Silicates and nitrog. matter	80·8	—	—	90·7
Coarse sand	7	40	21	81

to each of them, except the Wye soil, for the purpose of the present experiments.

The soils were air-dried till they appeared to be in a similar condition of dryness, when they were found to contain, Ridgmont 15·8, Wye 6·9 and Millbrook 4·0 per cent. of water. They were all heated at the same time in the autoclave, and in the germination experiments 5 c.c. of water were added to each 15 grams of the Ridgmont soil, and 4 c.c. to each 15 grams of the others, these quantities seeming to produce the same degree of moistness.

The germination experiments with these soils were all made at the same time, so as to be strictly comparable; and a series with the unheated Harpenden soil was also made, as well as one in which moistened blotting paper was used in the place of soil. The percentage of seeds germinated lead to no definite conclusions, and the mean results only are given in

TABLE XI. *Germination of seeds in soil from different localities.*

Temperature of heating	Germination percentage, relative	Relative times of incubation						
		Wheat	Rye	Clover	Mustard	*Lolium italicum*	*Festuca pratensis*	Mean
				Ridgmont soil				
Unheated	100	100	100	100	100	100	100	100
60°	101	107	213	106	89	107	121	129
100°	100	112	338	100	161	123	133	161
150°	93	114	229	222	200	123	131	170
				Wye soil				
Unheated	100	100	100	100	100	100	100	100
60°	99	102	128	90	145	111	79	114
100°	103	111	139	122	200	116	90	135
150°	105	117	114	157	245	111	90	140
				Millbrook soil				
Unheated	100	100	100	100	100	100	100	100
60°	105	107	139	111	85	106	85	106
100°	101	115	121	107	130	114	111	116
150°	105	105	150	107	200	140	114	135
				Harpenden soil				
Unheated	—	107	120	86	87	85	84	95
				Ridgmont soil				
Unheated	—	100	100	100	100	100	100	100
				Moist paper				
	—	103	126	116	96	98	91	105

Table XI; indeed, the percentage seems to be somewhat greater with the heated than with the unheated soils, the averages being

Unheated	*60°*	*100°*	*150°*
100	101·7	101·3	101·0 p.c.

but the run of these values is similar to that of the same seeds in Harpenden soil, the results from Table III giving

Unheated	*60°*	*100°*	*150°*
100	109	101	83 p.c.

although, when the results with other seeds are included, a diminution with rising temperature is observed throughout (p. 418).

The times of incubation, however, show a fairly steady increase in each case with the temperature of heating, this increase being greatest with the Ridgmont soil, and least with the Millbrook soil, though in all cases it is probably much less than with the Harpenden soil, where the values for the same seeds, according to Table III, would be

Unheated	*60°*	*100°*	*150°*
100	106	137	302 p.c.

A more striking illustration of the effect of heating the soil is obtained by setting out the percentages of seeds germinated within a given time; 24 or 48 hours, according to the different seeds.

Percentage of seeds germinated in 24 *or* 48 *hours.*

Soil	*Unheated*	*60°*	*100°*	*150°*
Ridgmont	58	45	33	8 p.c.
Wye	50	43	27	13 p.c.
Millbrook	58	49	36	26 p.c.

In Table XII the values for soils heated to each different temperature are treated separately, the results being expressed in relation to the Millbrook soil, taken as 100.

TABLE XII. *Times of incubation of seeds in different soils, heated and unheated.*

Soil	Times of incubation, relative values					Increase of organic matter by heating to 150°
	Unheated	Heated to 60°	Heated to 100°	Heated to 150°	Average of heated soil	
Ridgmont	88·5	105	112·5	115	110·8	·632
Wye.....	96·5	102·5	107	99	102·8	·335
Millbrook	100	100	100	100	100	·142

In the last column are entered the percentages of organic matter rendered soluble by heating to 150°, the details on which these last quantities are based being given in Table XIII, together with the incubation periods for the unheated soils, and also the results for the Harpenden soils and those for water (wetted blotting-paper).

From Table XII it is evident that, in the unheated state, the Ridgmont soil is the most favourable for germination, and the Millbrook soil the least so; but that, after heating, this order is exactly reversed, the reversal becoming more and more marked as the temperature of heating is higher. Further, the extent of the unfavourable effect produced by heating is more or less proportional to the amount of organic matter rendered soluble by the heating (last column), proving that what held good for the same soil when heated to different temperatures, holds good also for different soils heated to the same temperatures.

On looking at the more detailed results with the unheated soils (including that from Harpenden) in Table XIII, it is evident that, in their case, the results are the reverse, and that the time of incubation of seeds in them is less in proportion as the amount of soluble organic matter in the soil is greater.

TABLE XIII. *Times of incubation of seeds in unheated soils, and composition of soil extracts.*

Soil	Incubation times, relative	Composition of extract			
		Unheated soils		Soils heated to 150°	
		Organic	Mineral	Organic	Mineral
Harpenden.....	84·5	·315	·266	·845	·423
Ridgmont	88·5	·167	·128	·799	·250
Wye.....	96·5	·075	·137	·410	·145
Millbrook	100	·078	·075	·220	·093
(Water	93·5	0	0	—	—)

The general conclusion from these results must be, that the soluble organic matter in the soils examined is directly favourable to germination, and the more there is of it present, the more readily do seeds germinate[1]; but that on heating, it, or some of the other organic constituent in

[1] It has also been found, in the case of the last three soils in Table XIII, that this also holds good as regards the formation of adventitious roots by trees.

them, becomes converted into a compound which is inhibitory towards germination, and the richer and more favourable the soil was before heating, the more inhibitory does it become after heating.

The results with water, as will be seen from Table XIII, do not fall quite into line with those with soils: if the organic matter in unheated soils is uniformly favourable to germination, every soil should act more favourably than water alone; but the results with water assign to this latter a place intermediate between the Ridgmont and Wye soils. The strict conclusion from this would be, that, whilst some soils contain matter which renders them favourable to germination, others contain matter which is slightly inhibitory, rendering them less favourable for germination than water alone. This, certainly, is not improbable, in view of the fact that the inhibitory substance appears to be formed at temperatures as low as 30°; but it can hardly be affirmed on the strength of these determinations: for, apart from the fact that the difference on which the conclusion would rest approximates dangerously in magnitude to the experimental error, it is questionable whether the physical conditions in the case of seeds sown *on* moistened paper are sufficiently akin to those of seeds sown *in* moistened soil to warrant any rigorous comparison of the two[1]. Silica would have been a more suitable substance to use than paper.

The question of the existence of a toxic substance in soils is one of considerable importance in view of work recently done in America on this subject. In that case, toxicity towards growth, not towards germination, was investigated, but the two phenomena are, no doubt, intimately connected, and should be studied together. Whether the substance formed on heating soils is toxic towards plant growth as it is towards germination, is an open question at present, for the fact that plant growth is not adversely affected by heating the soil may be due to the substance becoming modified in the damp soil, before the plant reaches the stage of active growth. But the formation of it, whether it becomes altered or not, involves a large increase of soluble nitrogen in the soil, and this in itself may be quite sufficient to account for the increased growth and increased nitrogen assimilation observed.

Looking again at Darbishire and Russell's results in the light of the present work, further doubt must arise as to how far bacteria

[1] In connexion with this point it may be mentioned that germination in water vapour takes place more slowly than in the presence of liquid water. Some seeds placed on a platinum gauze tray suspended over water took 2·37 and 1·07 times as long to germinate, in the case of clover and *Festuca pratensis*, respectively, as they did on wet paper.

have yet been proved to be concerned in the matter. With heated soils, there is an increase of bacterial contents and of the oxygen absorption up to about 95°, followed by a rapid decrease in both, even to the extinction of the former at 120°, and it is clear that the incubation period of seeds cannot be primarily conditioned by either of these circumstances, for it increases continuously throughout this range, and even beyond it up to 200°. On the other hand the increase is directly proportional throughout to the extent of the chemical change brought about by the heating—the conversion of organic and nitrogenous matter into a soluble form—and such proportionality appears to extend even to the extreme limits of a red heat. The increase in the growth of plants follows precisely the increase in the incubation times of seeds, and, equally with the latter, it does not harmonise with the alterations in the oxygen absorption or bacterial conditions of the soil, but it does harmonise with those of the soluble nitrogen contents, although mathematical proportionality has not yet been established[1].

With soils treated with antiseptics we have—with the exception of the increase in soluble nitrogen, which has not been examined—every condition and effect exhibited by soils which have been heated, but in a minor degree, such treated soils being analogous to soils heated to about 60°. For attributing the increased growth in these treated soils, to those very conditions which are evidently inadequate in the case of heated soils, the only ground at present is that the antiseptics used could not have affected the soils chemically. This is a point on which we can scarcely speak with certainty, and the fact should be proved or disproved by direct experiment. The extent of the change sufficient to explain the results would, indeed, be very small. Judging by the values entered in Tables VI and X of Darbishire and Russell's paper, the excess of growth of plants grown in treated soil and in soil heated to 92°·5, is about 29 and 215 per cent., respectively: if the increase in growth follows the same course as the increase in the time of incubation of seeds, as given in the diagram on p. 419, the treated soil would be

[1] The additional nitrogen assimilated by the trees and plants in these experiments can be fully accounted for, so far as can be judged by the very imperfect data available, by the increase in the soluble nitrogen produced by heating. But it does not follow that these two quantities should be identical. Nitrogen is being rendered soluble during the growth of the plant, and if a plant gets a good start early in its life, owing to the richness of soil, or other circumstances, it may assimilate a greater excess of nitrogen than could be accounted for by the excess available in the soil to start with. This is certainly so in the case of trees, where the behaviour throughout life is largely conditioned by the growth when young.

analogous to soil heated to 57°. The chemical alteration at this temperature is too small, it is true, to be determined by analyses such as those quoted in Table V, but there should be no difficulty in increasing their accuracy indefinitely, by increasing the quantities of material taken.

SUMMARY.

Soil which has been heated without drying to temperatures from 60° to 150° behaves unfavourably towards the germination of seeds, the total seeds germinating decreasing (in most cases), and the time necessary for their germination increasing, with the temperature of heating. The average results are sufficiently regular to show that the alteration in the soil must begin at temperatures as low as about 30°.

Sterilised seeds behave in the same way as unsterilised ones, except that the time required for germination is uniformly longer. This is due to an alteration in the seeds by the sterilising agent, and not to the destruction of bacteria, for sterilised seeds do not recover their property of ready germination on being re-inoculated. Various sterilising agents were examined, but mercuric chloride was the only one found to be satisfactory and efficient.

The retarding effect on germination produced by heating the soil cannot be explained by an alteration in the bacterial condition of the soil, for the alteration extends progressively at temperatures beyond that sufficient to destroy all bacteria: moreover, no similar results are obtained with sand, even when this has been thoroughly impregnated with soil-bacteria to start with; also, re-inoculating previously sterilised seeds has no effect on their germination; and many seeds, after sterilisation, will germinate freely in a sterile medium.

By heating the soil, an increase in its soluble constituents occurs, especially in the soluble organic and nitrogenous matter, and the increase in either of these has been found to be directly proportional, within the limits of experimental error, to the increase in the time required for germination. The latter increase appears, therefore, to be due to the formation of a nitrogenous compound in the soil, which is inhibitory towards germination. This compound is sufficiently stable in solution for an extract of heated soil to affect an unheated soil when it is added to this latter: it also does not seem to be destroyed when the soil containing it is kept at a low atmospheric temperature for some months; but at a higher temperature, and in the presence of sufficient moisture,

it generally loses some of its inhibitory properties, probably through oxidation.

The inhibitory substance is not of an acid nature.

Soils from different localities favour the germination of seeds to different extents, the extent varying (in the three instances examined) directly with the amount of soluble organic matter in the soil: but, on heating these soils to the same temperatures, their action is exactly reversed, the soil richer in soluble organic matter forming, on heating, a larger proportion of inhibitory matter, and becoming, therefore, less favourable to germination.

The experiments do not settle definitely whether any of the inhibitory substance is ever present in soils before artificial heating, but it seems probable that such is the case.

The temperature of heating at which the inhibitory substance is formed in greatest quantity is about 200°: it diminishes in amount as the temperature is further raised, till it disappears entirely at a low red heat, such burnt soil behaving in nearly the same way towards germination as does unheated soil.

The substance which is inhibitory as regards germination need not necessarily be so as regards plant growth, or it may become destroyed before growth becomes active. Its presence results in an increase in the soluble nitrogen in the soil, and this may be the chief, if not the sole, reason of the increase of growth of non-leguminous plants in heated soil. Preliminary experiments with apple-trees have led to similar results, there being a large increase in growth, in leaf-formation, and in the percentage of nitrogen in the leaves, when the trees were grown in heated soil. The increase, also, was greater as the temperature of heating had been higher.

The heated soils, however, behaved towards the starting into growth of the tree in the same way as they did towards the germination of seeds, this starting into growth having been considerably delayed by the heating.

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